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EFFECT OF SUNLIGHT ON THE NITRIFICATION OF AMMONIUM SALTS IN SOILS

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Texas Agricultural Experiment Station

Received for publication March 2, 1934

In two recent publications, Dhar, et al. (2, 6) claim that the process of nitrification in the soil, especially in the tropics, is more a photochemical than a bacterial process. Other claims have recently been made that nitrification by simple physico-chemical action takes place in soils (1) and that photochemical nitrification occurs in sea water (7). The claim that nitrification is more photochemical than bacterial is very interesting to the soil investigator, since up to the present time nitrification has been generally accepted as a process carried out almost entirely by the action of specific microörganisms requiring certain favorable conditions in order to support their activity. A vast amount of experimental nitrification work has been successfully done in which the cultures received no sunlight whatever.

In a previous publication (4), evidence was presented to show that the low nitrification capacity of some Texas soils was due either to insufficient numbers of nitrifying organisms or to lack of calcium carbonate present in the soil, or to both. These experiments were all carried out in darkness in electrically heated incubators. The photochemical aspects of the problem, as claimed by Dhar et al., presented a new phase which required investigation. Accordingly, work was carried out at College Station, the results of which are presented in this paper.

PLAN AND METHOD OF WORK

The nitrification tests were carried out on six samples of soil from widely separated parts of Texas. Some information regarding these soils is given in table 1. One portion of the original soil was used without treatment, another was inoculated to increase the number of the nitrifying organisms originally present, a third portion was sterilized, and a fourth was sterilized and inoculated. Each was used both with and without ammonium salts. In each case one set was exposed to direct sunshine, while the other set was placed beside it, but protected from the light. The plan of the experiment is shown in tables 2 and 3. Portions of 200 gm. of air-dry soil were mixed with 10 cc. of a solution of ammonium salt shown by analysis to contain 0.10 gm. nitrogen, equal to 500 p.p.m. nitrogen in the dry soil, and sufficient water to equal 50 per cent of the water capacity of the soil. A similar preparation was made without any addition of ammonium salt. After a thorough mixing, the soil was transferred to

300-cc. tall Pyrex beakers, packed by striking the beaker on the palm of the hand, weighed, and placed outdoors during the day. The beakers exposed to the sunlight were covered with window glass in order to keep the dust out. Corresponding cultures were kept in the dark, being placed at the side of the exposed cultures but covered with galvanized iron cans. The ammonium salts used were reagents of tested purity.

For the preparation of the inoculating liquid which was added to some of the cultures, 20 gm. of soil from a soil culture was placed in a mortar with a few cubic centimeters of water and ground thoroughly to a thin paste. More water was added, a little at a time, with grinding, the mixture transferred to a 500-cc. volumetric flask, and made up to volume. The soil suspension was thoroughly shaken immediately before the aliquot of 10 cc. used for each culture was taken out.

The soils were sterilized by heating 200-gm. portions in 300-cc. tall Pyrex beakers, covered with watch glasses, in an electric oven for 2 hours at 140°–

TABLE 1

Description of soils

SOIL NUMBER	TYPE NAME	COUNTY	pH	DEPTH	BASICITY	NITROUS N	NITRIC N
				inches	*per cent*	*p.p.m.*	*p.p.m.*
9691	Houston black clay, subsoil	Wise	7.56	8 to 14	18.00	0	1.5
23094	Houston black clay (probably)	Dallas	7.20	0 to 7	4.91	0	9.4
32922	Victoria fine sandy loam (probably)	Karnes	7.67	Surface	5.01	0.3	16.3
36370	Shamrock very fine sandy loam	Wheeler	7.91	0 to 2	4.26	0.2	1.5
36473	Clareville clay loam	Bee	7.84	7 to 20	2.00	0.1	1.8
36498	Houston black clay	Bell	7.51	Surface	10.00	0.03	29.4

150°C. This procedure was found by previous tests to destroy the nitrifying organisms. The spatulas and porcelain dishes used for each culture were also sterilized.

The temperatures were taken daily, except Sunday, between 3:00 and 4:00 p.m. by means of a thermometer in one of the cultures that was exposed in the sunlight, and from another thermometer in one of those that were kept in the dark.

The cultures placed in the sun lost water more rapidly than did those left in the dark, but sufficient water to restore the loss in weight was added daily to all cultures. In order to expose the soil to the light as completely as possible, all cultures were thoroughly stirred with spatulas daily before the water was added.

After 28 days, each culture was weighed, emptied into a dish, mixed thoroughly, and an aliquot of one-tenth taken for the determination of nitrates and nitrites. The determinations of nitrates were made by the phenol-disulfonic acid method, and those for nitrites by the alpha-naphthylamine method (3).

TABLE 2

Net amounts of ammonia nitrogen changed to nitrates and nitrites in sunlight and in the dark

LAB. NUMBER OF SOIL	CONDITION OF SOIL	IN PARTS PER MILLION OF DRY SOIL						IN PERCENTAGE OF ADDED AMMONIA					
		$(NH_4)_2SO_4$		NH_4Cl		$(NH_4)_2HPO_4$		$(NH_4)_2SO_4$		NH_4Cl		$(NH_4)_2HPO_4$	
		Sunlight	Dark	Sunlight	Dark	Sunlight	Dark	Sunlight	Dark	Sunlight	Dark	Sunlight	Dark
9691	Unsterilized	0	98	0	0	0	109	0	20	0	0	0	22
9691	Unsterilized + inoculated liquid	0	217	0	31	1	475	0	43	0	6	0	95
9691	Sterilized	0	8	0	0	0	0	0	2	0	0	0	0
9691	Sterilized + inoculated liquid	0	86	0	0	8	119	0	17	0	0	2	24
23094	Unsterilized	0	278	0	133	19	358	0	56	0	27	4	72
23094	Unsterilized + inoculated liquid	0	411	0	228	54	474	0	82	0	46	11	95
23094	Sterilized	0	0	0	0	0	0	0	0	0	0	0	0
23094	Sterilized + inoculated liquid	11	45	0	134	24	173	2	9	0	27	5	35
32922	Unsterilized	0	419	0	235	14	484	0	84	0	47	3	97
32922	Unsterilized + inoculated liquid	0	434	3	324	56	477	0	87	1	65	11	95
32922	Sterilized	0	1	4	9	0	2	0	0	1	2	0	0
32922	Sterilized + inoculated liquid	1	9	0	270	18	227	0	2	0	54	4	45
36370	Unsterilized	0	394	0	155	358	515	0	79	0	31	72	103
36370	Unsterilized + inoculated liquid	0	411	0	409	336	527	0	82	0	82	67	105
36370	Sterilized	0	125	0	0	0	4	0	25	0	0	0	1
36370	Sterilized + inoculated liquid	0	90	0	33	19	29	0	18	0	7	4	6
36473	Unsterilized	0	440	0	160	187	405	0	88	0	32	37	81
36473	Unsterilized + inoculated liquid	0	452	0	387	247	471	0	90	0	77	49	94
36473	Sterilized	0	0	0	0	0	1	0	0	0	0	0	0
36473	Sterilized + inoculated liquid	0	231	2	0	5	3	0	46	0	0	1	1
36498	Unsterilized	124	476	0	163	0	495	25	95	0	33	0	99
36498	Unsterilized + inoculated liquid	181	495	0	294	15	515	36	99	0	59	3	103
36498	Sterilized	1	10	0	23	0	1	0	2	0	5	0	0
36498	Sterilized + inoculated liquid	32	220	0	239	6	76	6	44	0	48	1	15
Average		15	223	0.4	134	57	248	3	45	0.08	27	11	50

To avoid any interference of chloride with the determination of nitrates in the cultures which had received ammonium chloride, the chloride was precipitated with silver sulfate. The aliquot of the filtrate was evaporated; the residue after cooling was saturated with 5 cc. of 0.10 per cent silver sulfate solution;

TABLE 3

Effect of sunlight on the nitrification of ammonium sulfate and of soil nitrogen

(Nitrogen in parts per million)

LAB. NUMBER	CONDITION OF THE SOIL	WITH $(NH_4)_2SO_4$				WITHOUT $(NH_4)_2SO_4$				SUNLIGHT	CLOUDINESS	AVERAGE TEMPERATURES	
		Sunlight		Dark		Sunlight		Dark					
		Nitric N	Nitrous N	Nitric N	Nitrous N	Nitric N	Nitrous N	Nitric N	Nitrous N			Sunlight	Dark
										hours	*hours*	°C.	°C.
9691	Unsterilized	1	0	90	80	1	0	25	47	143	20	42.6	39.3
9691	Unsterilized + inoculated liquid	2	0	237	102	2	0	90	32				
9691	Sterilized	0	0	0	0	0	0	0	0				
9691	Sterilized + inoculated liquid	1	0	19	144	1	0	11	66				
23094	Unsterilized	25	16	341	0	28	20	63	0	148	15	42.9	39.7
23094	Unsterilized + inoculated liquid	16	7	479	0	22	11	67	0				
23094	Sterilized	0	0	0	0	0	0	0	0				
23094	Sterilized + inoculated liquid	10	0	0	72	0	0	0	27				
32922	Unsterilized	17	7	475	0	25	12	53	0	164	6	43.2	40.4
32922	Unsterilized + inoculated liquid	12	5	485	0	15	8	51	0				
32922	Sterilized	0	0	0	0	0	0	0	0				
32922	Sterilized + inoculated liquid	1	0	6	10	0	0	3	4				
36370	Unsterilized	50	10	500	1	122	3	107	0	164	6	43.2	40.4
36370	Unsterilized + inoculated liquid	9	0	430	88	38	3	107	0				
36370	Sterilized	0	0	30	95	0	0	0	0				
36370	Sterilized + inoculated liquid	1	0	36	136	1	0	18	64				
36473	Unsterilized	20	1	475	0	31	7	35	0	148	15	42.9	39.7
36473	Unsterilized + inoculated liquid	4	0	487	0	12	4	35	0				
36473	Sterilized	0	0	0	0	0	0	0	0				
36473	Sterilized + inoculated liquid	1	0	60	256	1	0	76	9				
36498	Unsterilized	171	31	533	0	38	0	37	0	130	30	41.6	36.4
36498	Unsterilized + inoculated liquid	239	23	538	0	38	3	33	0				
36498	Sterilized	3	0	5	7	2	0	2	0				
36498	Sterilized + inoculated liquid	11	34	83	210	6	7	71	2				

this was evaporated to dryness; phenol-disulphonic acid was added; and the work was completed as usual.

EFFECT OF SUNLIGHT ON OXIDATION OF AMMONIA

The cultures exposed to light in the summer and early fall received from 130 to 164 hours of bright Texas sunlight, the remainder of the period of exposure

being cloudy. Details of the work are given in tables which will be discussed later. A summary of the results relating to the production of nitrates and nitrites (combined) from the ammonium salts added, as affected by sunlight is given in table 2. In order to measure the oxidation of ammonia, the nitrogen oxidized in the soil alone was subtracted from the nitrogen oxidized in the

TABLE 4

Effect of sunlight on the nitrification of ammonium chloride and of soil nitrogen

(Nitrogen in parts per million)

LAB. NUMBER	CONDITION OF THE SOIL	WITH NH_4Cl				WITHOUT NH_4Cl				SUNLIGHT	CLOUDINESS	AVERAGE TEMPERATURES	
		Sunlight		Dark		Sunlight		Dark					
		Nitric N	Nitrous N	Nitric N	Nitrous N	Nitric N	Nitrous N	Nitric N	Nitrous N			Sunlight	Dark
										hours	*hours*	°C.	°C.
9691	Unsterilized	2	5	12	43	2	6	24	35	153	15	39.6	38.5
9691	Unsterilized + inoculated liquid	16	2	128	10	18	8	107	0				
9691	Sterilized	0	0	0	0	0	0	0	0				
9691	Sterilized + inoculated liquid	1	0	14	88	2	5	112	25				
23094	Unsterilized	39	12	201	3	44	13	71	0	149	22	40.8	39.4
23094	Unsterilized + inoculated liquid	38	2	303	0	49	7	75	0				
23094	Sterilized	0	0	0	0	0	0	0	0				
23094	Sterilized + inoculated liquid	0	3	251	6	0	1	123	0				
32922	Unsterilized	61	3	297	1	63	2	63	0	149	22	40.8	39.4
32922	Unsterilzed + inoculated liquid	52	2	385	0	45	6	61	0				
32922	Sterilized	4	0	4	6	0	0	1	0				
32922	Sterilized + inoculated liquid	6	0	355	0	8	0	85	0				
36370	Unsterilized	39	13	268	2	122	1	115	0	153	15	39.6	38.5
36370	Unsterilized + inoculated liquid	35	1	525	1	117	8	117	0				
36370	Sterilized	0	0	0	0	0	0	0	0				
36370	Sterilized + inoculated liquid	0	0	105	90	4	5	152	10				
36473	Unsterilized	2	0	190	3	11	4	33	0	153	15	39.6	38.5
36473	Unsterilized + inoculated liquid	1	0	420	0	7	3	33	0				
36473	Sterilized	0	0	0	0	0	0	0	0				
36473	Sterilized + inoculated liquid	0	4	5	37	2	0	16	76				
36498	Unsterilized	13	18	236	0	36	32	83	0	149	22	40.8	39.4
36498	Unsterilized + inoculated liquid	14	8	381	0	29	25	87	0				
36498	Sterilized	0	0	4	29	6	0	5	5				
36498	Sterilized + inoculated liquid	2	0	381	1	5	0	143	0				

corresponding cultures which received the ammonium salts. On an average, the ammonia in the sulfate was oxidized to the extent of 15 p.p.m. in the sunshine and 223 in the dark; in the chloride, 0.4 in the sunlight and 134 in the dark; in the phosphate, 57 in the sunshine and 248 in the dark. The average percentage of the ammonia oxidized was, for the sulfate, 3 in the sunlight and

45 in the dark; for the chloride, 0.08 in the sunlight and 27 in the dark; for the phosphate, 11 in the sunlight and 50 in the dark. The average for all cultures is 24 p.p.m. of soil, oxidized in the sunshine, compared with 202 p.p.m. in the dark, or 5 per cent of the ammonia oxidized in the sunshine compared with 41 per cent in the dark. These averages include all cultures, both sterilized and

TABLE 5

Effect of sunlight on the nitrification of ammonium phosphate and of soil nitrogen

(Nitrogen in parts per million)

LAB. NUMBER	CONDITION OF THE SOIL	WITH $(NH_4)_2HPO_4$				WITHOUT $(NH_4)_2HPO_4$				SUNLIGHT	CLOUDINESS	AVERAGE TEMPERATURE	
		Sunlight		Dark		Sunlight		Dark					
		Nitric N	Nitrous N	Nitric N	Nitrous N	Nitric N	Nitrous N	Nitric N	Nitrous N			Sunlight	Dark
										hours	*hours*	°C.	°C.
9691	Unsterilized	6	14	168	73	4	16	70	62	155	21	39.5	38.1
9691	Unsterilized + inoculated liquid	41	7	750	0	33	14	250	0				
9691	Sterilized	0	0	0	0	0	0	0	0				
9691	Sterilized + inoculated liquid	3	9	215	54	1	3	148	2				
23094	Unsterilized	44	10	421	0	27	8	63	0	150	20	39.5	38.3
23094	Unsterilized + inoculated liquid	98	2	541	0	41	5	77	0				
23094	Sterilized	0	0	0	0	0	0	0	0				
23094	Sterilized + inoculated liquid	22	7	266	5	0	4	93	5				
32922	Unsterilized	57	3	535	0	43	3	51	0	150	20	39.5	38.3
32922	Unsterilized + inoculated liquid	100	3	535	0	43	4	58	0				
32922	Sterilized	5	0	7	0	5	0	5	0				
32922	Sterilized + inoculated liquid	28	10	297	5	13	7	71	4				
36370	Unsterilized	460	8	612	0	110	0	97	0	155	21	39.5	38.1
36370	Unsterilized + inoculated liquid	450	8	625	0	122	0	98	0				
36370	Sterilized	0	0	2	6	0	0	4	0				
36370	Sterilized + inoculated liquid	132	38	90	115	128	23	176	0				
36473	Unsterilized	210	17	440	0	40	0	35	0	155	21	39.5	38.1
36473	Unsterilized + inoculated liquid	295	0	520	0	48	0	36	13				
36473	Sterilized	0	0	0	0	0	0	0	0				
36473	Sterilized + inoculated liquid	5	3	88	1	2	1	44	42				
36498	Unsterilized	28	15	571	0	31	24	76	0	150	20	39.5	38.3
36498	Unsterilized + inoculated liquid	53	7	594	0	34	9	81	0				
36498	Sterilized	6	0	9	0	8	0	8	0				
36498	Sterilized + inoculated liquid	8	0	103	51	2	0	61	46				

unsterilized. The differences would be greater if the sterilized cultures were omitted. In not a single one of the 72 comparisons between sunlight and no sunlight given in table 2, on six soils, with four treatments and with three salts of ammonia, is there any indication of any oxidation of ammonia due to sunlight. In many cases, there is no oxidation at all in the cultures exposed to

sunlight. Where oxidation occurs, it is invariably greater in the cultures kept in the dark than in those in the sunshine. The light is therefore detrimental to nitrification and not beneficial.

Similar results are, for the most part, to be observed in the results with the cultures of the original soils to which no ammonium salts were added (tables 3, 4, and 5). In 48 of these cultures more nitrification is found in those kept in the dark than in those exposed to sunlight. In 18 there was no difference. There are, however, 7 cultures of the unsterilized soil exposed to sunshine in which slightly more nitrates are found than in the corresponding cultures kept in the dark. This difference ranges from 8 to 18 p.p.m. in five of the six unsterilized cultures of soil 36370 (tables 3, 4, and 5), and from 1 to 8 p.p.m. in two cultures of soil 36498 in table 3. Most of the sterilized cultures of the same two soils and the sterilized cultures which were uninoculated with bacteria nitrify more in the dark than in the sunshine, and none of them nitrify more in the sunshine. The apparent slight photonitrification may be partly due to analytical errors but it is more probably due to irregularities in bacterial action; otherwise, it should be observed in the sterilized cultures of the same soils just mentioned. The possibility of a slight photochemical nitrification is not excluded by this work, but the possible amount is so slight in comparison with the bacterial action that it is practically of no significance.

EFFECT OF KIND OF AMMONIUM SALT USED ON THE OXIDATION

Dhar et al. (2) claimed that with 700 hours sunshine, 89 per cent of the nitrogen in ammonium phosphate, 80 per cent of that in ammonium chloride, and 13 per cent of that in ammonium sulfate were oxidized, while from 0 to 4.8 per cent was nitrified in the dark. He claimed that in 160 hours 12.2 per cent of the ammonium phosphate, and 6.7 per cent of the ammonium chloride were oxidized, while in the dark the amount was 0 to 1.5 per cent.

A comparison of these three salts is made in table 2, and additional details are given in tables 3, 4, and 5. The figures in the latter tables are the total amounts of nitrous and nitric nitrogen in the cultures at the end of the test. The quantities in the soil alone must be deducted and the nitric and nitrous nitrogen added together in order to get the quantities produced from the ammonium salts as given in table 2.

Tables 2 and 4 show that practically no oxidation occurred in any of the cultures containing ammonium chloride exposed to the sunshine. Oxidation of ammonium sulfate in the sunlight occurred in 5 of the 24 cultures (tables 2 and 3). Oxidation of diammonium phosphate occurred in the sunlight in most of the unsterilized soils. The average for 24 tests in the sunshine is 3 per cent of the nitrogen of ammonium sulfate oxidized, 0.1 per cent of that of ammonium chloride, and 11 per cent of that of diammonium phosphate. This apparently confirms Dhar's claim that ammonium phosphate oxidized to a greater extent in the sunshine than did the ammonium chloride or ammonium sulfate. In the dark, the oxidation—45 per cent, 27 per cent, and 50 per cent for sulfate,

chloride, and phosphate respectively—was much greater than in the sunshine. Thus the difference in the oxidation of the ammonium sulfate and of the ammonium phosphate appears to be really due, not to greater oxidation by the sunlight, but to a lower inhibiting effect of the light on bacterial activity, possibly because the ammonium phosphate cultures were exposed to less intensive light than were the cultures containing the other two salts.

EFFECT OF SUNSHINE ON NITRIFYING ORGANISMS

If photonitrification occurs, it should take place in sterilized soils. Tables 2, 3, 4, and 5, show that no nitrification occurred either in sunshine or in darkness with most of the sterilized samples: the amount of oxidation is only a few parts per million where it occurs, with the exception of one culture of soil, No. 36370. This culture was probably not completely sterilized. The amount of nitrates produced in the few other sterilized samples was small and may have been due to contamination, during the period of exposure, with dust which would be mixed into the soil during the daily stirring.

Nitrification occurred in all the cultures which were kept in the dark and contained the nitrifying organisms, either originally in the soil or added by the inoculating liquid. Evidently the nitrifying organisms produced the nitrification. The cultures kept in the sun invariably produced less nitrates than did the corresponding cultures kept in the dark. The sunlight decreased the activity of the nitrifying organisms and reduced the nitrification. Apparently the sunlight either killed the organisms or prevented them from multiplying. According to W. M. Gibbs (5), cultures of both nitrosomonas and nitrobacter are so sensitive to light that when inoculated in cultures in the laboratory neither organism will produce oxidation unless protected from light; direct sunlight causes complete destruction of the organisms, whereas diffused light merely hinders their activity unless the period of exposure is long, which proves fatal.

EFFECT OF SUNSHINE ON NITROUS AND NITRIC ORGANISMS

The discussion so far has concerned only the total oxidation of the ammonia. Both nitrous and nitric nitrogen were estimated, as shown in tables 3, 4, and 5. It is generally accepted that ammonia must pass through the nitrite stage before it is converted to nitrates. If such is the case, both the nitric and nitrous organisms must have been destroyed, or prevented from developing, by sunlight, since the sum of the nitric and nitrous nitrogen is much less in the cultures exposed to light than in those kept in the dark.

Tables 3, 4, and 5 show that a number of cultures exposed to the light contain a little more nitrites than do the corresponding cultures protected from light. In some cases, no nitrites occur in the cultures kept in the dark but they occur in appreciable amounts in the cultures exposed to sunlight. It appears from these results that the nitrous organisms are more resistant to light than are the nitric organisms. The difference is small, however, since the quantities of nitrites produced in the cultures exposed to light are small.

DISCUSSION

It must be pointed out that the temperature (taken daily at 3–4 p.m. except Sunday) was higher in the cultures exposed to sunlight than in the covered ones near by. The average differences (tables 3, 4, and 5) were from 5.2 to 3.2°C. for the cultures containing ammonium sulfate exposed in May, June, and July, but the average differences were only about 1 to 1.5° for those containing ammonium chloride and ammonium phosphate exposed in September and October. The temperature in all cases was higher than is believed to be the optimum (35°C.); nevertheless, nitrification was high in many of the cultures where the organisms were present and which were protected from the sun.

The work here presented does not entirely exclude the possibility of a slight photochemical oxidation of ammonium salts in the soil during long periods of sunlight. It shows, however, that under the conditions described at College Station, Texas, the photochemical oxidation, if it occurs, is vastly inferior in importance to the nitrification caused by organisms. It also shows that direct sunlight is injurious to the microörganisms. The activity of the nitrifying organisms may be decreased or entirely eliminated on soil surfaces exposed to direct sunlight. The surface exposed to light is, however, relatively very small compared to the mass of the soil, so that the injurious action of light upon bacterial nitrification is probably insignificant from a practical point of view.

SUMMARY

To determine how light affects nitrification, samples of soils exposed to sunlight for 4 weeks and stirred daily to present new surfaces to the light were compared with corresponding samples protected from the light. On an average of 72 comparisons conducted on six soils, each with four treatments and three different salts of ammonia, 5 per cent of the ammonia was oxidized in sunlight and 41 per cent in the dark. The oxidation of the added ammonium salt was in all comparisons lower in the cultures exposed to the light than in corresponding cultures that were kept in the dark.

The decrease in nitrification in the sunlight was lower with ammonium phosphate than with ammonium chloride or ammonium sulfate, though this may have been due to the exposure's being made at a period when the light was less intense than when the other two soils were exposed. Nitrites in small amounts were found in cultures exposed to the light, whereas little nitrites or none was found in the cultures kept in the dark, all the nitrogen oxidized being changed to nitrates. This indicates that the nitric organisms are less resistant to light than are the nitrous organisms.

Both nitrous and nitric organisms were rendered inactive or were destroyed by sunshine in cultures of soils exposed to sunlight and stirred daily.

If photonitrification occurs, it should take place in sterilized soils. No evidence of nitrification due to sunlight was found in any of the sterilized soils.

A few of the unsterilized cultures of soils exposed to sunlight and to which ammonium salts were not added contained slightly more nitrates or nitrites

than did the corresponding soils kept in the dark, but sterilized samples of the same soil, as well as sterilized soils inoculated with nitrifying organisms, did not contain more nitrates when kept in the sun than when kept in the dark. The increases in the few cultures mentioned were probably due to differences in bacterial activity and not to photonitrification.

Although this work does not entirely exclude the possibility of photonitrification, it shows not only that photonitrification must be of little or no practical importance but also that sunlight greatly decreases the nitrification caused by bacterial action, when the bacteria are directly exposed to the sunlight. Since only a small portion of the soil is exposed to direct sunlight, the destructive action of sunlight upon nitrifying organisms is not likely to be of agricultural importance.

REFERENCES

(1) DeRossi, G. 1933 Nitrification in soils by simple physicochemical action. (Bol. Soc. Internatl. Microbiol., Sez. Ital, 5: 132–136) *Chem. Abs.* 27: 5860.

(2) Dhar, N. R., Bhattacharya, A. K., and Biswas, N. N. 1933 Photonitrification in soil. *Soil Sci.* 35: 281–284.

(3) Fraps, G. S., and Sterges, A. J. 1931 Estimation of nitric and nitrous nitrogen in soils. *Tex. Agr. Exp. Sta. Bul.* 439: 1–22.

(4) Fraps, G. S., and Sterges, A. J. 1932 Causes of low nitrification capacity of certain soils. *Soil Sci.* 34: 353–363.

(5) Gibbs, W. M. 1919 The isolation and study of nitrifying bacteria. *Soil Sci.* 8: 427–471.

(6) Rao, G. G., and Dhar, N. R. 1931 Photosensitized oxidation of ammonia and ammonium salts and the problem of nitrification in soils. *Soil Sci.* 31: 379–383.

(7) Zo Bell, C. E. 1933 Photochemical nitrification in sea water. *Science* 77: 27–28.

CHARACTERISTICS OF CERTAIN BACTERIA BELONGING TO THE AUTOCHTHONOUS MICROFLORA OF SOIL[1]

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In an article published in 1917 (3) the senior author discussed the great abundance of non-spore-forming bacteria in soil, especially of those which were designated at that time as "slow-growers." Their significance in soil was then stated to be problematic but undoubtedly important. Since then special attention has been given in this laboratory to this group of bacteria, and two or three subsequent papers have been published concerning them. In these later papers the original term "slow-growers" has been dropped because it has been realized that the bacteria do not grow slowly in soil. Instead, they have been called the "punctiform-colony formers."

DISCUSSION OF PREVIOUS WORK

Meanwhile, Winogradsky resumed his work in soil bacteriology and, beginning his investigations with a microscopic study of soil, noticed the large number of organisms present that did not seem to grow on his plates. A few translations from one of his first papers on this subject (9) are quite to the point:

> In studying the progress of decomposition of organic matter in the soil, one comes to distinguish two successive periods, as well as two great groups of organisms which correspond to them, or which preside over them.
>
> The first is characterized by relatively rapid activities of which the successive phases gradually grow more slowly as the organic molecule becomes less and less liable to attack by the majority of the ferments. The turn of the second group comes promptly after the organic matter, enriched by unutilized carbon, is transformed into a brown or black substance which is an intimate part of arable soil. The course of the phenomenon is then definitely changed. It assumes the character of slow combustion, much studied by the soil chemists, but of which soil biologists have not yet taken up the study.
>
> One already knows a considerable number of bacterial species belonging to the first group; as they lend themselves well to culture in the conventional media rich in nitrogenous material, they have been isolated without difficulty. The current methods, based exclusively on cultural technique, do not reveal the others which are to be regarded as the types actually representing the soil microflora.
>
> Now, in following the origin of the former and their activity, one concludes that they penetrate periodically into the soil from outside with the organic matter in the course of decomposition (bodies of animals, plant residues, buried manure, etc.), and that they do nothing

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in soil except carry on the activities already under way, favored only by the protection which they find there, by the humidity, and by the bases which they can borrow therefrom; but they live only at the expense of their preferred nutrients, recently incorporated with the soil, and they carry out their activities only as long as the latter last, after which they pass into a resting stage, which can continue indefinitely in the soil.

If one, therefore, decides (which seems logical) to consider as *biologically normal* a soil which does not contain any organic substance in the course of decomposition, nor in general any fermentable material, such as proteins or amino compounds, carbohydrates, higher alcohols, organic acids, etc. (i.e., no other substances as organic food except the brown or black material), then one can conclude that the group in question will be completely inactive; it cannot be a part of the microflora of active soil.

By the direct method which we have recommended, which is based on the microscopic study of soil, it is easy to prove this point of view. The most careful microscopic examination of normal soil, indeed, does not reveal in the vegetative condition any microörganisms active upon the substances cited above: neither bacilli (which one believes so characteristic of good soil) nor yeasts, nor fungi, etc. These organisms are present only in resting stages which, sometimes on account of their limited number and sometimes by their minute size and difficult stainability, generally escape observation in the microscopic study of the soil.

If one wishes to make them multiply, it is only necessary to add to the soil an appropriate organic substance of the category which we have mentioned. The effect is immediate but temporary, for this multiplication stops and merely leaves in the soil a new reserve of resting forms as soon as the substances which have brought about their multiplication are exhausted.

These very simple experiments are most convincing. They show clearly that the activity of this group of microörganisms is distinctly associated with the presence of substances foreign to normal soil and consequently their activity can only be intermittent and interrupted as it is (so far as concerns field soil) by resting periods longer than those of activity.

Hence in leaving out of account this group of microörganisms, widespread in most media but not in that of the soil, one comes to believe that the true microflora of normal arable soil is still unknown and that for this reason it is necessary to study the agents of slow combustion so characteristic in this natural medium.

The application of the direct method, indeed, permitted us to discover at the very beginning of our research in the laboratory of l'Institut Pasteur de Brie Comte-Robert a number of species still unknown living in an arable soil not having received any fertilizer for several years. . . .

These rather numerous observations lead us to look at this group as consisting of *"organismes humicoles"* especially adapted, therefore, to the medium in which they live; and it is these to which we refer in grouping them under the designation of the *autochthonous microflora* of arable soil.

In these same papers (9, 10) Winogradsky indicates his intention of doing more extensive work on the *autochthonous microflora* of soil. His interests of recent years, however, have been confined largely to other groups of bacteria, and his later publications, therefore, have not dealt with this particular group. It is, accordingly, concluded that he is still uncertain just what the nature of these bacteria may be. In his early papers he refers to them as organisms that cannot be cultivated on ordinary culture media. He assumes this because of the fact that they are largely round or nearly round organisms which he calls "cocci," while very few of the cultures obtained from soil on ordinary media are coccus forms.

The observation a few years later (5) as to the great abundance in soil of *Bacterium globiforme* Conn tends to make it a little less certain that the or-

ganisms seen under the microscope by the direct technique cannot be obtained by cultural methods. This organism, as shown in its original description, appears as a rod for the first day or two after being transferred to a fresh medium, but subsequently becomes spherical, and practically always appears in soil as a coccus. This organism may, therefore, well comprise a large part of Winogradsky's *autochthonous microflora*. Furthermore, the writer ventures to differ to some extent as to the prevalence of the spherical forms, at least so far as concerns the soils examined in the United States. Among the soils studied here by the direct method, very small rods, so short as to resemble cocci, seem to be more numerous than truly spherical forms. Furthermore, many of the small rods and coccoid bodies seen in such preparations are actually *Actinomycetes* spores. These latter are practically indistinguishable from bacteria in such preparations unless one stains slides that have been in actual contact with undisturbed soil, as did Rossi (7) and Cholodny (2), in which preparations the spores of these organisms can be recognized by their orientation.

For all of these reasons there seems some question about Winogradsky's conclusions that none of the *autochthonous microflora* have been isolated by cultural methods. As a matter of fact, it seems entirely probable that these organisms are for the large part identical with the group previously described in this laboratory as "slow-growers" or the "punctiform-colony formers."

PROBABLE RELATIONSHIPS OF THE ORGANISMS

The present paper is written on the assumption that the *autochthonous microflora* discussed by Winogradsky consists of *Bact. globiforme* and other closely related forms which in soil resemble cocci. It is, accordingly, of interest to consider what the relationships of these organisms to other bacteria may be. As long as it was assumed that they were forms that could not be isolated from soil, no study could be made of their relationships to other bacteria. Assuming, however, that they correspond to the aforementioned group of bacteria that have been isolated, a systematic study of them becomes possible.

The organisms considered in this publication are non-spore-forming organisms, and during recent years several efforts have been made to classify them. The earliest classification, which has since been regarded as unsatisfactory, was on the basis of gelatin liquefaction and chromogenesis. Finally in 1925 (4) it was shown that the bulk of these organisms consisted of two groups, the first simple rods, and the second what were then called "coccus-forming-rods." Not much progress has been made since that date in classifying the bacteria, although in 1928 it was concluded (5) that the second of these two groups consists of only one organism, *Bacterium globiforme*, which appears as a rod in cultures only 1 or 2 days old but as a coccus in older cultures.

The classification of these bacteria is difficult because they have so few features by which they can be characterized. The simple rods differ very little from one another in morphology or apparently in their physiology. The or-

dinary bacterial tests, such as ability to ferment various sugars, do not give satisfactory results when applied to these bacteria, because of the difficulty in obtaining end products in measurable quantities. Thus, all of these bacteria seem to utilize various sugars and to produce a small amount of acid therefrom; but the amount of acid is so small that it can almost never be detected in a highly buffered medium and, even when it is studied in a synthetic medium, its appearance is such an irregular phenomenon that it hardly proves a satisfactory basis for characterization.

This being the case, the writers are still uncertain whether to call all the simple rods that produce punctiform-colonies on gelatin, members of a single species or to consider that there is a great variety of such bacteria in soil. The greatest probability seems to lie with the latter assumption, but just how many species there may be or how they differ from one another is still a puzzling question.

It is likewise uncertain just where to place these organisms in any of the generally accepted classifications of bacteria. Before the peculiar morphology of *Bact. globiforme* was discovered it was assumed that some of these bacteria belonged among the cocci and some among those short rods that have been gathered together in Bergey's Manual (1) under *Achromobacter*. As a matter of fact, the latest edition of this manual places *Bact. globiforme* in the aforementioned genus. This name for it is not accepted by the writers, however, as it is felt that further study of this and similar organisms must be made before deciding whether to put them closest to the rods or the cocci. The fact that *Bact. globiforme* grows in soil in a spherical form suggests close relationships to the cocci. On the other hand, the similarity of this organism in its physiology to the other punctiform-colony forming bacteria of soil (and in many respects, indeed, to the legume nodule organisms) suggests relationships to the *Bacteriaceae*.

On account of this puzzle, the writers prefer to retain the name originally given to this organism. This is done in recognition of the fact that no such genus as *Bacterium* is recognized in Bergey's Manual and that, accordingly, an organism whose relationships are doubtful might well remain in the old genus *Bacterium* until it has been more carefully studied.

It is largely to give it this needed study that *Bact. globiforme* has received such a large share of the writers' attention in recent years; but there are other reasons also for the intensive study that has been given the organism. When first observed it appeared interesting because of its apparent ability to change back and forth from a rod to a coccus form. Still greater interest was added to it when it was discovered that none of the cultures on hand in the writers' laboratory had been isolated from sod soils, although among the simple non-spore-forming rods in the same collection of cultures as many had been isolated from sod as from cultivated soil. Even more significant was the observation that this organism appeared to be more abundant in the better soils than in those of low productivity. This last observation suggested that *Bact.*

globiforme either had some causal relation to the productivity of soil, or else those conditions favoring the growth of plants likewise stimulate the development of this organism.

It has since been found that *Bact. globiforme* in its physiology is quite typical of the whole group of punctiform-colony formers in soil. This fact, together with its peculiar morphology which makes it easy to recognize, seems to indicate this organism as a good one to be selected as a typical representative of the group under investigation.

FUNCTION OF THE ORGANISMS

In calling the bacteria which predominate in normal soil "autochthonous (i.e., *indigenous* or *native*) microflora," Winogradsky has certainly selected a much more appropriate name than either of those previously employed by the writer. In referring to them, however, with the adjective "*humicoles*" or "*humivores*" (10), it is not so certain that his terminology is correct. These two latter terms indicate, respectively, "living on humus" or "feeding on humus." In proposing these names, therefore, he is indicating the functions of the organisms in question in spite of the fact that he has not isolated them or learned their behavior. There is, on the other hand, an entirely different function that such organisms as these might perform and yet appear in exactly the conditions under which Winogradsky finds them.

This other possible function is as follows: In the course of the active decomposition of organic matter such as is carried on by the other group of organisms (called by Winogradsky *zymogenous*), much soluble nitrogen is formed as well as the insoluble brown or black material on which Winogradsky assumes the autochthonous organisms feed. This soluble form of nitrogen consists largely of nitrates, ammonium salts, and amino compounds. It is entirely possible, therefore, that a group of organisms may exist in the soil which seize upon these soluble forms of nitrogen and build them up into body protein. Inasmuch as all arable soil is quite sure to be supplied with a constantly renewed source of soluble compounds of this sort, it would be a fairly constant source of food for such organisms as these, and hence they would be uniformly present with much less fluctuation in numbers than the organisms of the zymogenous type.

Organisms carrying on the activities just mentioned would, of course, require in addition to this soluble nitrogen some source of carbon as well as a source of energy. A source of both carbon and energy might well be found in the humic constituents upon which Winogradsky assumes them to live. On the other hand, they might utilize for this purpose, either directly or indirectly, the celluloses, hemicelluloses, or starches occurring in soil. If they utilize such carbohydrates indirectly, it must be assumed that they live in association with the cellulose-decomposing bacteria and obtain their carbon and energy from the products of the hydrolysis brought about by these other forms.

With this thought in mind it becomes of interest to review the facts so far

known as to the activities of *Bact. globiforme* and the other punctiform-colony bacteria in soil to see how well they could play the aforementioned rôle. Although the function of these organisms is still a matter of speculation, certain inferences can be drawn from their distribution and from their nutritive requirements.

Especially significant in this connection are the results previously brought out (5) indicating those nitrogen sources available to *Bact. globiforme.* It was shown in the previous publication to be capable of obtaining its nitrogen from nitrate, ammonium salts, amino compounds, or even higher forms of nitrogen such as those present in bacteriologic peptones. Apparently it is capable of utilizing almost any source of soluble nitrogen, either organic or inorganic. This fact has been established both by culture in ordinary laboratory media and by observing which nitrogen compounds enable it to grow vigorously in soil which does not naturally contain any nitrogen available for the organism. In other words, so far as concerns its nitrogen relationships, this organism and presumably the others under investigation might well play the rôle discussed in the foregoing.

As to sources of carbon and energy available to the organism, the work mentioned has shown that stimulation of its growth can occur in soil upon the addition of certain organic acids without other added carbon compounds. Probably, therefore, such compounds can furnish bacteria of this group with their carbon and energy. Most readily available, however, of all carbon sources prove to be the sugars, especially glucose. At first thought it seems strange that those bacteria apparently the most numerous in soil should have a predilection for the simple sugars which are never found in soil. On further consideration, however, the situation is not so unlikely as it might seem. The decomposition of cellulose, pentosans, and starch is occurring constantly in the soil, and at an early stage of this decomposition there is almost sure to be production of some hexose or pentose. That these lower carbohydrates do not accumulate in soil may well be because such organisms as those here discussed are always present to consume the sugars as rapidly as they are formed. Thus, organisms capable of utilizing minute quantities of sugar may well find their place in Nature's scheme for the carbon and nitrogen transformations in soil.

Assuming this to be the function of the organisms under investigation, an intensive study seemed called for to learn just how economically they are able to utilize the simple sources of carbon and of nitrogen available to them. This was the object of the work discussed in the following. In this work glucose or sucrose was chosen as a source of carbon, and ammonium phosphate as a source of nitrogen. Analyses were made to determine to what extent the nitrogen and the carbon in these compounds were utilized for building cell tissue and for the production of energy.

Three cultures were used in this work: a strain of *Bact. globiforme* which had been kept as a stock culture in the laboratory since the species was first de-

scribed; also two undetermined simple short rods here denoted only by the laboratory numbers L $2i_4$ and III $2i_8$. These last two cultures were included largely for purpose of comparison with *Bact. globiforme.*

Utilization of sugars

In table 2 of the 1928 publication on this subject (5) are given certain preliminary figures as to the amount of sugar consumed in a liquid medium containing 1 per cent glucose. The results then obtained were noticeably discrepant. It was concluded that the discrepancies were caused by the difficulty of determining sugar accurately when the difference between the initial quantity and the amount left after the growth of the culture is small. In order to obtain a higher percentage of sugar consumed, an experiment was carried on to determine the minimum quantity of glucose that could be added to a culture medium and still permit approximately the maximum of growth and production of carbon dioxide. This work was done in Eldredge tubes containing

TABLE 1

CO_2 production in liquid medium with varying percentages of glucose

Incubation—14 days

CULTURE	TEST NUMBER	CO_2 PRODUCED FROM MEDIA CONTAINING GLUCOSE IN PERCENTAGES AS FOLLOWS*						
		0.02 per cent	0.05 per cent	0.1 per cent	0.2 per cent	0.5 per cent	1 per cent	1.5 per cent
		mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
Bact. globiforme	1		10.5	17.5	23.5	25.2	**27.9**	
Bact. globiforme	2	3.6	10.4†	15.9	**22.1**	19.5†	18.9	17.2
Unidentified short rods L $2i_4$	3	5.0	9.7	13.3	10.5	13.0	**15.3**	14.0
Unidentified short rods III $2i_8$	4	3.5	11.7	**19.7**	14.1	16.3	11.5†	12.5

* All determinations in triplicate except those marked †, which were in duplicate only.

20 ml. of medium in one arm and the same quantity of 0.1 *N* barium hydroxide in the other. The basal medium employed was as follows:

$(NH_4)_2HPO_4$	1.0 gm.
KCl	0.2 gm.
$MgSO_4 \cdot 7H_2O$	0.2 gm.
H_2O	1,000.0 ml.

To this basal medium were added different quantities of glucose from 0.02 per cent up to 1.5 per cent. The results of the four tests which were run for this purpose are given in table 1. In the line recording the results of each test the maximum CO_2-production is indicated in bold face type. It will be seen that the percentage of sugar giving the maximum amount of CO_2 varied considerably in the different tests, as it was occasionally as high as 1 per cent and in one instance as low as 0.1 per cent. It is noticeable, however, that very little tendency to increase was observed with concentrations of glucose greater

than 0.1 per cent. As a result of these figures it was concluded that 0.1 per cent or 0.2 per cent would be about the best quantity to employ in order to obtain the highest percentage of sugar utilization. As a matter of fact, the quantity employed in the following experiments was 0.15 per cent.

In connection with these results it is interesting to compare the respiration figures recently published by Georgi and Wilson (6) concerning the legume nodule organism. In this work they employed an agar medium in Eldredge tubes and determined not only the amount of carbon dioxide given off but the amount of oxygen consumed. Of special interest is the fact that, with quantities of glucose varying from about 0.6 per cent up to about 1.6 per cent, they found the amount of carbon dioxide produced to vary from about 10 to 20 ml. (e.g., from 20 to 40 mgm.) for each tube. Such results are similar to those obtained in the present work with a different kind of soil bacteria. Georgi and Wilson found that between 60 and 80 per cent of the carbon in the glucose consumed was converted into carbon dioxide.

The experiments which follow indicate how nearly these last-mentioned figures of Georgi and Wilson agree with results obtained with *Bact. globiforme* and the related organisms in soil.

In seven different experiments Eldredge tubes were prepared with 25 ml. of the medium in the culture arm and in the other arm 15 ml. of 0.1 *N* barium hydroxide diluted with distilled water up to 20 ml. The cultures were incubated for 2 weeks, and the barium hydroxide was then titrated to determine the amount of CO_2 given off. The contents of the culture arm were then placed in a centrifuge for about 2 hours, after which the supernatant fluid was decanted from the centrifuge tube and a sugar determination made of it by the macro method of Shaffer and Hartmann (8). Meanwhile, the growth which remained in the centrifuge tube was washed in the centrifuge two or three times, then removed, dried, and weighed. It was desired by this procedure to obtain the following information: amount of sugar utilized; percentage of the carbon in that sugar converted into CO_2; amount of carbon utilized in building up the bacterial cells, on the assumption that the growth obtained on centrifuging was mostly protein and contained approximately 50 per cent carbon; and finally, the percentage of carbon in the sugar not accounted for in either of these ways. In connection with the aforementioned assumption of 50 per cent carbon in the dried bacterial growth, it should be remarked that this was obtained from the estimate that if this substance were all protein it would contain about 53–54 per cent carbon. Presumably, however, a certain amount of carbohydrate and a certain amount of ash would be present, so that no accurate estimate as to the percentage of nitrogen in the cell substance can be made; but as the quantities of material other than protein were probably not large, the 50 per cent assumption seemed a safe one to make. It was not expected, however, that by any means all the carbon could be accounted for in these experiments; even if the organisms were capable of utilizing the glucose without the production of by-products, it would be very unlikely that

TABLE 2

Sugar consumption by Bact. globiforme

TUBE NUMBER	SUGAR CONSUMED			CO_2 PRODUCED		RATIO: $\frac{C \text{ IN } CO_2}{C \text{ IN SUGAR}}$
	Quantity	Per cent	C-content	Quantity	C-content	
Experiment 1. Initial sugar: 32.6 mgm. glucose per tube						
	mgm.	*per cent*	*mgm.*	*mgm.*	*mgm.*	*per cent*
1	19.7	60.4	7.9	26.0	7.1	90
2	28.2	86.5	11.3	16.1	4.4	39
3	20.6	62.2	8.25	19.8	5.4	65.5
4	23.1	70.8	9.25	20.7	5.65	61
5	24.2	74.3	9.7	18.5	5.05	52
6	27.4	84.0	10.9	22.2	6.05	55.5
7	27.0	82.8	10.8	21.3	5.8	53.5
8	25.1	77.0	10.1	19.6	5.35	53
Total for 200 ml.....	**195.3**	**74.8**	**78.2**	**164.1**	**44.8**	**57.3**
Bacterial growth in 200 ml.: **40** mgm.; estimated C-content: **20** mgm. Total C accounted for: **64.8** mgm. = **83** per cent of C consumed						
Experiment 2. Initial sugar: 37.5 mgm. glucose per tube						
1	7.5*	20	3.0	19.4	5.3	
2	33.1	87.2	13.2	22.9	6.25	
3	30.9	82.2	12.4	19.1	5.2	
4	3.0	8	1.2	19.4	5.3	
5	17.0	45.3	6.8	18.7	5.1	
6	36.7	97.7	14.6	23.8	6.5	
7	12.7	33.8	5.1	18.7	5.1	
8	36.7	97.7	14.6	18.9	5.25	
Total for 200 ml.....	**177.6**	**59.2**	**70.9**	**160.9**	**44.0**	**62.1**
Bacterial growth in 200 ml.: **48** mgm.; estimated C-content: **24** mgm. Total C accounted for: **68** mgm. = **97.5** per cent of C consumed						
Experiment 3. Initial sugar: 39.6 sucrose per tube						
1	24.0	60.7	9.6	15.8	4.3	44.8
2	18.1	45.8	7.25	14.4	3.9	53.8
3	25.2	68.8	10.1	19.1	5.2	51.5
4	16.1	40.8	6.45	13.9	3.8	59.0
5	14.1	35.7	5.65	13.9	3.8	67.3
6	16.6	42.0	6.65	17.8	4.85	73.0
7	17.0	43.0	6.8	15.8	4.2	61.8
8	20.9	52.9	8.4	13.9	3.8	45.3
Total for 200 ml.....	**152.0**	**48.0**	**60.9**	**124.6**	**33.9**	**55.7**
Bacterial growth in 200 ml.: **52.7** mgm.; estimated C-content: **26.4** mgm. Total C accounted for: **60.3** mgm. = **99** per cent of C consumed						

TABLE 2—*Concluded*

TUBE NUMBER	SUGAR CONSUMED			CO_2 PRODUCED		RATIO: C IN CO_2 / C IN SUGAR
	Quantity	Per cent	C-content	Quantity	C-content	
Experiment 4. Initial sugar: 33.2 glucose per tube						
	mgm.	*per cent*	*mgm.*	*mgm.*	*mgm.*	*per cent*
1	11.6	35.0	4.65	13.7	3.7	79.5
2	13.6	41.0	5.45	13.4	3.65	67.0
3	7.4	21.3	2.9	10.3	2.8	96.5
4	7.7	24.2	3.1	10.8	2.95	95.0
5	9.2	27.7	3.7	11.9	3.25	87.8
6	12.0	36.2	4.8	14.1	3.85	80.5
7	8.5	25.6	3.4	11.2	3.05	89.5
8	12.0	36.2	4.8	14.7	4.0	83.5
Total for 200 ml.....	82.0	30.8	32.8	100.1	27.25	83.1

Bacterial growth in 200 ml.: 34.0 mgm.; estimated C-content: 17 mgm.
Total C accounted for: 44.2 mgm. = 135 per cent of C consumed

* The great variation in the individual determinations in this experiment are thought to be analytical errors. See text.

all the growth could be separated from the medium and, therefore, all the carbon thus utilized determined.

The results of these experiments are given in tables 2 and 3—table 2, those with *Bact. globiforme*, and table 3, those with the unindentified short rods. Before any conclusions are drawn, it seems well to mention instances where the work may possibly be in error. In experiment 2 of table 2, for example, the sugar analysis was made under conditions which caused extreme variation, as shown by the figures given. It was planned at first to discard the results of this experiment entirely; but the average figures proved to agree so well with those of other experiments that they are given for whatever they may be worth. In the second place, experiment 4 of table 2 showed an unusually low figure for sugar consumption in every instance, so low, in fact, that apparently 135 per cent of this figure was accounted for in the products of growth; although no other evidence of analytical mistakes was obtained in this experiment, it is plain from the results that some such error must have occurred. Finally, it must be explained that experiments 1 and 3 of table 3 were carried on with a slime-producing organism, and it is known that some of this slime was lost in the course of washing the cells, and accordingly no estimate could be made as to its weight and carbon content.

Even when these possibilities of error are taken into account, the figures do bring out certain points of significance. It will be seen, for example, that the amount of sugar utilized, leaving out experiment 4 with *Bact. globiforme*, varied only from 48 to 74.8 per cent of that initially present. This agrees very closely with the 60 to 80 per cent found by Georgi and Wilson in the

TABLE 3

Sugar consumption by unidentified short rods

TUBE NUMBER	SUGAR CONSUMED			CO_2 PRODUCED		RATIO: C IN CO_2 / C IN SUGAR
	Quantity	Per cent	C-content	Quantity	C-content	
Experiment 1. Culture no. III 2i₈. Initial sugar 34:0 mgm. glucose per tube*						
	mgm.	*per cent*	*mgm.*	*mgm.*	*mgm.*	*per cent*
1	19.8	58.2	7.9	12.6	3.44	43.5
2	18.1	53.2	7.25	12.6	3.44	47.4
3	15.2	44.7	6.1	10.6	2.89	47.3
4	13.5	39.7	5.4	10.8	2.95	54.6
5	15.5	45.5	6.2	10.8	2.95	42.6
6	16.7	49.1	6.7	10.1	2.76	41.2
7	13.8	40.6	5.5	10.3	2.81	56.1
†Total for 200 ml.....	**128.7**	**47.3**	**51.5**	**88.9**	**24.3**	**47.2**
Bacterial growth in 200 ml.: 40 mgm.; estimated C-content: 20 mgm. Total C accounted for: **44.3** mgm. = **86** per cent of C consumed						
Experiment 2. Culture no. L 2i₄. Initial sugar 33.4 mgm. glucose per tube						
1	18.3	54.8	7.3	16.05	4.4	60.3
2	16.7	50.0	6.7	14.75	4.05	60.5
3	17.6	52.6	7.05	13.85	3.8	53.9
4	20.6	61.7	8.25	14.5	3.95	47.8
5	16.7	50.0	6.7	14.95	4.1	61.3
6	23.0	68.9	9.2	17.6	4.8	52.2
7	16.4	49.0	6.55	14.5	3.95	60.3
8	16.4	49.0	6.55	13.4	3.65	55.7
Total for 200 ml.....	**145.7**	**54.5**	**58.3**	**119.6**	**32.7**	**56.1**
Bacterial growth in 200 ml.: **60.4** mgm.; estimated C-content: **30.2** mgm. Total C accounted for: **62.9** mgm. = **107.8** per cent of C consumed						
Experiment 3. Culture no. III 2i₈. Initial sugar 34.1 mgm. glucose per tube*						
1	15.2	44.5	6.1	16.5	4.5	73.8
2	19.8	53.1	7.95	15.6	4.25	53.5
3	16.5	48.4	6.6	13.4	3.65	55.3
4	17.1	50.2	6.85	15.2	4.15	60.6
5	15.1	44.3	6.05	14.3	3.9	64.5
6	15.2	44.5	6.1	14.5	3.95	64.8
7	17.1	50.2	6.85	13.0	3.55	51.8
8	14.8	43.4	5.9	14.3	3.9	66.2
Total for 200 ml.....	**130.8**	**47.9**	**52.40**	**116.8**	**31.85**	**60.8**
Bacterial growth in 200 ml.: **24.3** mgm.; estimated C-content: **12.1** mgm. Total C accounted for: **44** mgm. = **84** per cent of C consumed						

* A slime-producing organism. No estimate made of the amount of carbon in the slime.

† One tube of this series was lost but the figures in this line are computed on the basis of 8 tubes (i.e., 200 cc.).

case of the legume nodule organism. Also, the ratio of carbon in the CO_2 produced, to the carbon in the sugar utilized (also leaving out experiment 4, table 2) was never below 47.2 per cent or above 62.1 per cent. Georgi and Wilson found this ratio to be from about 40 per cent to about 90 per cent in the case of the organisms they were studying; and this gives further evidence as to similarities between the organisms here under investigation and those of the legume nodules. Finally, it will be seen from these tables that the total carbon accounted for amounted in every experiment to over 80 per cent of the sugar consumed.

The conclusion drawn from these data is that organisms of this punctiform-colony group probably utilize sugars for two purposes only. Part of the sugar is used as a source of carbon and is built up into their cell tissue. Part of it is used as economically as possible as a source of energy and for this purpose is oxidized completely into carbon dioxide. Any sugar that is present in excess of the needs of the organisms for these two purposes remains unchanged in the medium. Thus these bacteria are characterized by an extremely economical utilization of sugar.

One interesting corollary of this fact is that since no by-products are produced from the sugar, the only changes in reaction that can occur are from the CO_2 produced and from the action of bacteria on ingredients other than the carbohydrate. This makes it difficult to devise a simple test, such as acid production, by means of which the action of the bacteria on sugars can be observed. The acid formed from the CO_2 is likely to be insufficient to make itself evident unless the medium is quite free from buffer compounds. In the latter case, changes of reaction due to other causes (such as greater utilization of anions than cations, or *vice versa*) may be so great as to obscure production of acid from the sugar.

Utilization of ammonium salts

As has been stated, ammonium salts are among the preferred sources of nitrogen for *Bact. globiforme*. It became of interest, accordingly, to make an analytical study of the utilization of ammonium salts by this organism. Two experiments were conducted for this purpose. Ammonium phosphate was chosen and the organism was grown on the following medium:

$(NH_4)_2HPO_4$	2.0 gm.
KCl	0.4 gm.
$MgSO_4 \cdot 7H_2O$	0.4 gm.
Glucose	20.0 gm.
Agar (air-dried)	30.0 gm.
H_2O	2,000.0 gm.

The medium was distributed among several containers in which it was allowed to harden in such a position that there would be as large a surface as possible to inoculate. About two-thirds of the containers were inoculated and the others were kept as checks. After 2 weeks of incubation the growth

was carefully removed from the surface of the agar and the nitrogen was determined by the modified Kjeldahl method of the Association of Official Agricultural Chemists. At the same time ammonia determinations were made (by distillation by magnesium oxide) on 200-gm. samples of the inoculated medium after the growth had been removed, and of the uninoculated medium which had been incubated under exactly the same conditions as the inoculated containers.

The object of these determinations was to learn what proportion of the ammonium phosphate could be utilized by this organism and what percentage of that which it did use was converted into cell tissue.

Experiment 1. In the first experiment the containers used for the medium were large test tubes about 30 mm. in diameter. In each tube 30 ml. of the medium was allowed to harden while in a slanted position. The total weight of the inoculated medium after incubation was 1,326 gm. Aliquots of this and of the uninoculated medium were analyzed, and all computations were figured to the basis of the entire 1,326 gm. The results of the analysis were as follows:

Ammonia N in uninoculated medium	239.3 mgm.
Ammonia N in inoculated medium	90.5 mgm.
Ammonia N consumed	148.8 mgm.
Dry weight of bacterial growth	1.6738 mgm.
Total N in bacterial growth	118.2 mgm.
Percentage of ammoniacal N consumed appearing as total N in growth	79.1 per cent

Experiment 2. The second experiment differed from the first in two respects. The containers employed were 500-ml. wide-mouth Erlenmeyer flasks, and 100 ml. of the agar was placed in each flask. The larger surface presented by the agar in these flasks made it much easier to remove the growth after incubation. The other point of difference was that it seemed worth while to make a total nitrogen analysis of the medium from which the growth had been removed. Accordingly, all of the inoculated medium (after the bacterial growth was removed) that was not used for the ammonia analysis was kept in tightly closed flasks in a refrigerator until the next analytical work could be carried on (about 3 weeks). The same method of nitrogen analysis was employed as in the case of bacterial growth, but the large bulk of agar made the digestion process a difficult one. It was first necessary to reduce the bulk of the medium somewhat by partly drying for several days (after adding a small amount of 5*N* H_2SO_4) at a temperature of 55°C. Six or seven weeks were required for the digestion process, as it was not possible to arrange to have digestion kept up continuously night and day. The analytical data obtained were as follows:

Ammonia N in uninoculated medium	183.5 mgm.
Ammonia N in inoculated medium	3.0 mgm.
Ammonia N consumed	180.5 mgm.
Dry weight of bacterial growth	2.4472 mgm.
Total N in bacterial growth	127.0 mgm.
Percentage of ammoniacal N consumed appearing as total N in growth	70.3 per cent
Total N in inoculated medium after removal of growth	108.8 mgm.
Total N in growth	127.0 mgm.
Total N in medium after growth	108.8 mgm.
Total N present after growth	235.8 mgm.

In this experiment the nitrogen balance was not completely figured out because no total nitrogen determination was made on the uninoculated medium. It was known, however, that it contained at the end of the experiment (as has been shown above) 183.5 mgm. ammonia nitrogen and that in addition to the ammonium salt no nitrogen was added to the medium except in the agar and in the way of impurities. On the basis of published analyses of agar it was concluded that at least 35 to 40 mgm. of nitrogen would be added to the medium from this source, so that a total of over 220 mgm. was undoubtedly present before the growth of the bacteria. This checks very closely with the 235.8 mgm. actually found in the inoculated medium after incubation. This at least indicates that none of the nitrogen was lost into the air.

In general it was concluded from these nitrogen analyses that this organism can utilize all the nitrogen present when this element is furnished in the form of 0.1 per cent ammonium phosphate. Nearly 80 per cent of the nitrogen is converted into cell tissue. The remaining portion of the nitrogen consumed, in the instance under investigation, remained in the medium, apparently in some soluble organic form. It is entirely possible that even the nitrogen not accounted for by the cell substance present at the end of the experiment may have been originally converted into cell protein and subsequently decomposed after death of some of the cells.

SUMMARY

A physiological study has been made of some of the organisms previously designated "punctiform-colony formers" because of the small size of their colonies on laboratory media. Reasons are given for considering them as belonging to the group which was seen but not isolated by Winogradsky and called by him the *autochthonous* (i.e., indigenous) *microflora* of soil.

Special attention has been given to one of these organisms, *Bacterium globiforme* Conn, because it is very easy to recognize and because it has been found more numerously in good soils than in those less productive.

In a synthetic liquid medium it has been found that under the conditions

investigated the highest percentage of glucose is utilized if the medium is furnished with about 0.2 per cent of this sugar.

When this medium with 0.15 per cent glucose or sucrose was used and the culture grown in 150-ml. Eldredge tubes containing 25 ml. of the medium, the organisms studied were found to utilize ordinarily between 48 and 75 per cent of the sugar furnished. About 50 to 60 per cent of the carbon in the sugar consumed was converted into CO_2. From the weight of the bacterial growth produced, an estimate was made of the amount of carbon that had been converted into cell substance. When this estimate was added to the amount of CO_2 produced, over 85 per cent of the carbon in the sugar consumed was accounted for. It is, accordingly, concluded that the organisms convert all the carbon of glucose or sucrose into CO_2 and cell substance.

As a similar conclusion has recently been drawn by Georgi and Wilson concerning the legume nodule organism, there seems to be some possibility that this extremely economical utilization of sugar is quite characteristic of a number of soil bacteria.

A study was also made of the nitrogen consumption of *Bact. globiforme* when growing on a solid medium containing 0.1 per cent ammonium phosphate as its sole source of nitrogen. It was found that 60 to 90 per cent of the nitrogen thus furnished was consumed, and that 70 to 80 per cent of that consumed was converted into cell substance. The balance of the nitrogen remained after growth in the medium, no loss of nitrogen being evident.

It is concluded that these bacteria undoubtedly help retain in soil, nitrogen that has been converted by other organisms into a soluble form and, but for the action of this autochthonous microflora, would probably be removed by drainage or utilized by plants. It is still uncertain whether to regard these organisms as ordinarily harmful or beneficial. Perhaps they may be either, according to the conditions under which they are functioning. At times they may be rivals for nitrogen because of their ability to utilize nitrate. On the other hand, under conditions that bring about rapid nitrification in the soil these bacteria may be beneficial. When ammonification is so rapid that ammonium salts tend to accumulate faster than they can be nitrified or nitrates are formed more rapidly than they can be utilized by the plants, there would be considerable loss of nitrogen by leaching but for the action of bacteria in converting it into an insoluble form.

Of these two functions it seems more probable that the beneficial action predominates, to judge from the fact that this group of organisms is ordinarily most abundant in the better soils.

Whether ordinarily harmful or beneficial, it is plain that the organisms studied are well adapted to live under surface soil conditions, as they are very strictly aerobic, can make use of nitrates, ammonium salts, or amino compounds as sources of nitrogen, and can obtain their carbon and energy from such small amounts of sugar that they can undoubtedly utilize for this purpose the minute quantities made available in soil during the digestion of starch and

cellulose. Utilizing simple sources of nitrogen and very small quantities of carbohydrate, they convert inorganic material into cell substance which, undoubtedly, is readily converted into humus upon the death of the bacteria.

The functions of these organisms in soil are evidently of some importance. Whether these activities can be controlled remains for future investigation to show.

REFERENCES

(1) BERGEY, D. H. 1930 Bergey's Manual of Determinative Bacteriology, ed. 3. Williams & Wilkins, Baltimore, Md.

(2) CHOLODNY, N. 1930 Über eine neue Methode zur Untersuchung der Bodenmikroflora. *Arch. Mikrobiol.* 1: 620–652.

(3) CONN, H. J. 1917 Soil flora studies. IV. Non-spore-forming bacteria in soil. N. Y. Agr. Exp. Sta. Tech. Bul. 59.

(4) CONN, H. J. 1925 Soil flora studies. VI. The punctiform-colony-forming bacteria in soil. N. Y. Agr. Exp. Sta. Tech. Bul. 115.

(5) CONN, H. J. 1928 A type of bacteria abundant in productive soils, but apparently lacking in certain soils of low productivity. N. Y. Agr. Exp. Sta. Tech. Bul. 138.

(6) GEORGI, C. E., AND WILSON, P. W. 1933 The influence of the tension of oxygen on the respiration of Rhizobia. *Arch. Mikrobiol.* 4: 543–564.

(7) ROSSI, G., AND RICCARDO, S. 1927 L'esame microscopico e batteriologico diretto del terreno agrario. *Nuovi Ann.* [*Italy*] *Min. Agr.* 7: 457–470.

(8) SHAFFER, P. A., AND HARTMANN, A. F. 1921 The iodometric determination of copper and its use in sugar analysis. II. Methods for the determination of reducing sugars in blood, urine, milk, and other solutions. *Jour. Biol. Chem.* 45: 365–390.

(9) WINOGRADSKY, S. 1924 Sur la microflore autochtone de la terre arable. *Compt. Rend. Acad. Sci.* [*Paris*] 178: 1236.

(10) WINOGRADSKY, S. 1925 Études sur la microbiologie du sol. 1. Sur la méthode. *Ann. Inst. Pasteur.* 39: 299–355.

INORGANIC PHOSPHATE IN GREEN PLANT TISSUE AS A MEASURE OF PHOSPHATE AVAILABILITY[1]

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In recent years a number of investigators (4, 5, 7, 9, 12, 13) have suggested that a determination of the content of inorganic or total phosphorus in the sap or tissues of growing plants might throw some light on the phosphate supply of the soil. Thornton (13) in particular has advanced the idea that plants growing in a medium richly supplied with phosphate may be unable to convert the inorganic phosphate into organic form as rapidly as it is absorbed; hence, inorganic phosphate will accumulate within the plant. Conversely, under conditions of deficient supply no such accumulation will occur.

Gilbert and Hardin (5), Emmert (4), Thornton (13), and Pohlman and Pierre (12) have found that, under certain conditions, phosphate fertilization, or an increased phosphate supply in the nutrient medium, causes an increase in the inorganic phosphate of the culture plants. Conversely, it has been shown that plants, when limited in growth by phosphate deficiency, contain reduced amounts of inorganic phosphate. However, none of the previous studies are sufficiently complete to show whether low inorganic phosphate in the tissues of the growing plant is always indicative of a low phosphate supply in the culture medium, and *vice versa*, whether high phosphate in the plant is definitely indicative of an adequate supply. Neither has it been determined definitely just what part of the plant and what stage of growth best reflect the phosphate condition of the nutrient medium, although a number of the aforementioned investigators have devoted some study to this question. In order to obtain further information on these points, pot culture experiments were made with oats on a series of soils varying with respect to phosphate requirement. In addition, a few preliminary tests have been made with citrus. It is the purpose of this paper to report the results of these studies.

EXPERIMENTAL

Method of making inorganic phosphate tests

The method finally adopted is a slight modification of that employed by Thornton (13). Immediately after withdrawal from the soil, the oat plants

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were stripped of dead leaf material, the roots were cut off at the crown, and the adhering soil was washed off with distilled water. The excess water was then absorbed between sheets of filter paper. Samples of 0.5 gm. of the part of the plant to be tested were immediately cut into small pieces, transferred to a small glass cylinder, and thoroughly macerated with a blunt glass rod. The macerated material was then transferred with acid-molybdate reagent (0.1 per cent ammonium molybdate dissolved in 0.4 N H_2SO_4) to a 250-cc. Erlenmeyer flask, and enough more of this reagent added to give a final volume of 100 cc. The contents of the flask were shaken vigorously, 10 drops (0.5 cc.) of a 2.5 per cent $SnCl_2 \cdot 2H_2O$ solution added, and after standing 2 minutes the color intensity was compared against Hellige glass color standards (1). In cases where the color was too intense, the solutions were diluted with the acid-molybdate reagent and more $SnCl_2$ solution was added. Chlorophyl introduces an error in the phosphate-molybdate color measurements, especially if the inorganic phosphate content is low; hence the results obtained with material containing relatively much chlorophyl and little phosphate are but roughly quantitative. The small quantities of inorganic constituents found in most plants are probably insufficient to interfere, provided the method is used as here outlined (2). No important interference by soluble organic constituents was found to occur with the particular plant materials studied.

It is not certain that this test distinguishes quantitatively between inorganic phosphate and certain forms of organic phosphorus. It is conceivable that in an acid medium PO_4 might be formed from certain organic forms by hydrolysis. However, Plimmer's (11) studies on the hydrolysis of nucleic acid, phytin, hexose phosphate, and simple alcoholic phosphates suggest that no appreciable hydrolysis of such of these compounds as may occur in the plant material tested is likely to take place under the conditions of this test. In any case, if an important relationship is found between the phosphate of the nutrient medium and the content of PO_4 in the plant or any of its parts, it is immaterial from a practical point of view whether the distinction between inorganic and organic phosphorus is absolute.

Emmert (3) has outlined a somewhat more exact but less rapid method, and Pierre and Pohlmann (10) have determined inorganic phosphate in the exuded sap of plants.

Pot culture studies with oats

A series of 17 soils varying with respect to phosphate need were assembled, and portions of each were screened, mixed, and potted in 2-gallon earthenware jars.

Duplicate pots of each soil were treated with fertilizers as shown in table 1. The fertilizers were mixed uniformly with the soil in a given pot, the soluble forms being applied in solution and dicalcium phosphate being added to the soil in dry form. In addition $Ca(NO_3)_2$, at the rate of 50 pounds per acre, was applied in solution to the N-treated pots at intervals of 2 weeks during

the growing period. Oats were planted, and when the seedlings had attained a height of 1 inch the stand was thinned to 20 plants per pot.

Different parts of the plants were tested for inorganic phosphate at three stages of growth; namely, the vegetative, heading, and ripening stages. The plants at the first (vegetative) stage were 24 to 26 days old and had attained a height of 6 to 8 inches. At the heading stage plants were chosen in which the panicles were emerging or had just emerged. At the ripening stage the plants were still somewhat green, and the grain was in the early dough stage. Ten plants per pot were used for making these tests, leaving ten plants to be harvested. When approximately mature, these were cut near the crown, dried, and weighed.

Effect of varying phosphate supply.—The oat yields resulting from the differential treatments are recorded in table 2. If it is assumed that sufficient fer-

TABLE 1

Different fertilizer treatments given each soil under pot culture

TREATMENT		GRAMS PER POT	POUNDS PER ACRE		
			N	P_2O_5	K_2O
None	.....	.....	.....	.....	.....
N	$Ca(NO_3)_2 \cdot 4H_2O$	1.44	50	.....	.....
NP	$Ca(NO_3)_2 \cdot 4H_2O$	1.44			
	$CaH_4(PO_4)_2 \cdot H_2O$	1.19	50	400	.....
	$CaHPO_4 \cdot 2H_2O$	1.62			
NPK	$Ca(NO_3)_2 \cdot 4H_2O$	1.44			
	$CaH_4(PO_4)_2 \cdot H_2O$	1.19	50	400	200
	$CaHPO_4 \cdot 2H_2O$	1.62			
	K_2SO_4	1.25			

tilizer was added to meet crop needs, the yield increase resulting from the NP treatment as compared with the N treatment measures the relative deficiency of P in the pots treated with N alone. In like manner, the relative deficiency of N and K is measured by the yield increases resulting from N as compared with no treatment and NPK as compared with NP. It will be noted that the yield increases produced by phosphate ranged from none to 115 per cent; by nitrogen, from 20.4 to 620.0 per cent; and by K, from no increase to 24 per cent.

Preliminary tests of various parts of the young plants at the first sampling stage showed higher inorganic phosphate in the primary stem and sheath material immediately above the crown, and in the young immature leaves, than in the more mature leaves. However, at this stage both the stem and leafy parts of the plant seemed to be consistent in reflecting the phosphate condition of the nutrient medium. Because of the interfering action of chlorophyl, the tests on leaves are less accurate than those on the stem-sheath ma-

terial immediately above the crown, hence only the results on the latter are recorded in table 3.

As has been stated, the yields resulting from the NP, as compared with the N treatments, afford a measure of the relative deficiency of phosphorus in the N-treated pots. Since the increases from phosphate additions ranged from nothing up to 115 per cent, a measure of the inorganic phosphate level in the oat plants growing in the N-treated pots to which no phosphate was added should indicate whether at this stage the plant will accurately reflect the phosphate supplying power of these soils. The data bearing on this point are recorded in table 3. It will be noted that there is a striking relationship between the

TABLE 2

Effect of fertilizer treatments on oat yields

SOIL NUMBER	SOIL TYPE	YIELDS RESULTING FROM FERTILIZER TREATMENT*				INCREASE IN YIELD FROM		
		None	N	NP	NPK	N	P	K
		grams	*grams*	*grams*	*grams*	*per cent*	*per cent*	*per cent*
18322	Meloland fine sandy loam	22.5	27.1	26.6	33.0	20.4	None	24.1
18313	Hanford sandy loam	4.4	31.4	30.0	26.4	620.0	None	None
18323	Sierra loam	5.6	23.4	23.5	22.1	318.0	0.4	None
18321	Yolo clay loam	11.7	33.6	34.1	31.9	187.0	1.5	None
18324	Ramona loam	5.8	24.8	25.2	26.1	318.0	1.6	3.5
18325	Placentia loam	5.2	21.6	22.0	18.1	316.0	1.8	None
18319	Yolo gravelly loam	4.5	19.3	19.9	21.7	329.0	3.1	9.0
18314	Hanford loam	4.9	24.0	26.7	28.5	390.0	11.2	6.7
18315	Hanford sandy loam	7.6	21.1	23.9	22.8	178.0	13.3	None
18327	Hanford fine sandy loam	5.1	20.6	24.4	21.1	304.0	18.4	None
18318	Ramona loam	7.0	18.2	27.0	27.3	160.0	48.3	1.1
18310	Yolo loam	8.8	18.5	28.4	29.7	110.0	53.5	4.0
18316	Ramona loam	11.4	16.0	27.0	28.4	40.5	69.0	5.2
18312	Yolo clay loam	5.0	16.1	28.5	29.5	222.0	77.0	3.5
18317	Indio sandy loam	6.7	13.2	23.5	27.6	92.0	78.0	17.4
18320	Chino clay loam	6.4	12.8	27.4	25.4	100.0	114.0	None
18311	Hanford sand	4.2	11.0	23.6	22.6	162.0	115.0	None

* Average dry weight (grams) of tops, per pot.

inorganic phosphate in the stem-sheath material and response to phosphate. Without exception, those plants which grew on soils substantially deficient in phosphate (growth increases ranging from 48 to 115 per cent) were very low in inorganic phosphate, whereas those which grew in soils showing little or no phosphate deficiency contained considerably more phosphate. A set of earlier results with oats in the vegetative stage grown in 11 other soils are recorded in table 4. These results are consistent with those of table 3.

That additions of phosphate to the nutrient medium will result in an increased accumulation of PO_4 in the plant is again shown by the data of table 5. It will be noted that in many cases phosphate applications caused markedly increased amounts of PO_4 in the plants. Since the plants grown on the first

eight soils of this series showed little or no response from phosphate fertilizer, the increases in inorganic phosphate within the plants resulting from phosphate additions represent "luxury" consumption.

It will be noted that applications of potassium sulfate slightly reduced the inorganic phosphate content of the plants when grown in 12 of the 17 soils (compare columns 4 and 5 of table 5). Whether this effect is due to decreased phosphate absorption, as such, or to more complete conversion of inorganic phosphate into organic forms within the plants, cannot be stated. On the other hand, with 4 other soils the application of K_2SO_4 caused the oat plants to accumulate increased amounts of PO_4. Thornton (13) found that potash

TABLE 3

Relationship between response to phosphate applications and the inorganic PO_4 content of green oat plants

(Vegetative stage—plants 26 days old)

SOIL NUMBER	INCREASE IN YIELD FROM PHOSPHATE ADDITIONS	INORGANIC PO_4 IN GREEN OAT PLANTS GROWN IN N-TREATED POTS (STEM-SHEATH MATERIAL 0–1 INCH ABOVE CROWN)
	per cent	*p.p.m.*
18313	None	368.0
18322	None	166.0
18323	0.4	129.0
18327	1.4	334.0
18321	1.5	722.0
18324	1.6	219.0
18325	1.8	306.0
18319	3.1	114.0
18314	11.2	165.0
18315	13.3	64.0
18318	48.3	46.0
18310	53.5	23.0
18316	69.0	53.0
18312	77.0	23.0
18317	78.0	46.0
18320	114.0	46.0
18311	115.0	23.0

fertilization was associated with increased concentrations of inorganic phosphate in corn plants.

Preliminary tests on oat plants at later stages of growth (see table 6) indicated, as in the vegetative stage, that the young growing parts of the oat plant contain higher concentrations of inorganic phosphate than do more mature parts. At the heading stage there is a pronounced gradient in the stem, more phosphate being found in the upper stem and rachis than in the lower parts of the stem. At this, and at the ripening stage, sections of both the lower stem and upper stem were analyzed. The results are recorded in table 7.

It will be noted that there is less correlation between the phosphate responses

and the inorganic phosphate of the plants at maturer stages of growth than at the younger vegetative stages. Although the lower stems of all the plants grown in phosphate-deficient soil were consistently low in inorganic phosphate, a corresponding condition was found in certain of the plants grown in soils that were not deficient in phosphate. Moreover, at the heading stage inorganic phosphate in the upper parts of the stems was in many cases as high where the plants were grown in phosphate-deficient soil as where grown in soil well supplied with phosphate. Apparently, at this stage of growth phosphate is rapidly translocated from the leaves and lower stems to the developing seed. The rate of absorption from the soil probably decreases as maturity approaches, also. However, major differences in the phosphate content of the nutrient media were still reflected in the inorganic phosphate of the plants at the head-

TABLE 4

Relationship between response to phosphate applications and the inorganic PO_4 content of green oat plants

(Vegetative stage—oats 45 days old)

SOIL NUMBER	SOIL TYPE	INCREASE IN YIELD FROM PHOSPHATE ADDITIONS	INORGANIC PO_4 IN GREEN OAT PLANTS GROWN IN N-TREATED POTS (STEM-SHEATH MATERIAL 0–1 INCH ABOVE CROWN)
		per cent	*p.p.m.*
18050	Hanford loam	None	191.0
18048	Placentia loam	14.7	55.0
18051	Hanford sand	48.5	34.0
18067	Kimball sandy loam	121.0	34.0
18066	Yolo clay adobe	131.0	14.0
18057	Yolo clay loam	162.0	13.0
18047	Hanford loam	195.0	11.0
18068	Hanford loam	210.0	11.0
18055	Yolo clay loam	263.0	Trace
18063	Yolo loam	320.0	11.0
18052	Chino clay loam	560.0	Trace

ing stage (N-treated pots as compared with NP-treated pots), but translocation and transformation processes have tended to obscure the more direct relationships noted in early growth stages. That the translocation of PO_4 from the stems to the seeds proceeds very rapidly is indicated by the greatly decreased phosphate concentration in the upper stem at the ripening stage as compared with that found at the early heading stages. In this later stage there is little, if any, correlation between phosphate supply and the inorganic phosphate content of the parts tested.

Effect of nitrogen deficiency upon inorganic phosphate accumulation.—Since all of the soils used in this experiment are more or less deficient in nitrogen, the amounts of inorganic phosphate found in plants grown with and without nitrogen fertilization should show the effect of nitrogen deficiency on the accumulation of inorganic phosphate in the plant. Data bearing on this point are recorded in table 8. They show definitely that nitrogen deficiency causes

inorganic phosphate to accumulate within the plant. In some cases the increases resulting from this cause are very striking. With plants grown in

TABLE 5

Effect of phosphate and potash applications upon inorganic phosphate content of green oat plants

(Vegetative stage—plants 24–26 days old)

SOIL NUMBER	INCREASE IN YIELD FROM PHOSPHATE ADDITIONS	INORGANIC PO_4 IN GREEN OAT PLANTS GROWN IN DIFFERENTIALLY FERTILIZED POTS (STEM-SHEATH MATERIAL 0–1 INCH ABOVE CROWN)		
		N treatment	NP treatment	NPK treatment
	per cent	*p.p.m.*	*p.p.m.*	*p.p.m.*
18313	None	368	890	668
18322	None	166	368	712
18323	0.4	129	503	445
18327	1.4	334	890	813
18321	1.5	722	890	654
18324	1.6	219	709	798
18325	1.8	306	989	797
18319	3.1	114	1,023	829
18314	11.2	165	712	712
18315	13.3	64	844	736
18318	48.3	46	859	668
18310	53.5	23	167	140
18316	69.0	53	973	890
18312	77.0	23	404	380
18317	78.0	46	219	252
18320	114.0	46	320	300
18311	115.0	23	167	184

TABLE 6

Distribution of inorganic phosphate in oat plant at heading stage

PART OF PLANT			INORGANIC PO_4 IN VARIOUS PARTS OF GREEN OAT PLANT	
			Soil 18313	Soil 18327
			p.p.m.	*p.p.m.*
Stem		Between uppermost node and rachis	406.0	611.0
		2 inches below uppermost node	170.0	231.0
		4–6 inches above crown	.....	83.0
		0–2 inches above crown	27.0	50.0
Leaves	Upper	Blade	.....	.....
		Sheath	184.0	.....
	Middle	Blade	Medium	Medium
		Sheath	.....	150.0
	Lower	Blade	Low	Low
		Sheath	83.0	61.0

phosphate deficient soils, little or no accumulation of inorganic phosphate occurred in the early growth stages, no doubt because the plants at this stage were suffering more or less equally from both nitrogen and phosphate defi-

ciency. At the heading stage, however, the accumulation of PO_4 was more evident.

Gilbert, McLean, and Adams (6), Thornton (13), and McCool and Weldon (8) have obtained similar results, the first named investigators having found that low nitrate may cause inorganic phosphate to accumulate in the plant, and conversely low phosphate causes the accumulation of nitrate. McCool and Weldon (8) state: "If one element is decidedly a limiting factor, the slow growth of the plant appears to permit the accumulation of high concentrations of other elements."

TABLE 7

Relationship between response to phosphate applications and the inorganic PO_4 in green oat plants

SOIL NUMBER	INCREASE IN YIELD FROM PHOSPHATE ADDITIONS	INORGANIC PO_4 IN STEMS OF OAT PLANT					
		Heading stage*				Ripening stage†	
		Fertilized with N only		Fertilized with NP		Fertilized with N only	
		Lower stem 0–2 inches above crown	Upper stem 0–4 inches below rachis	Lower stem 0–2 inches above crown	Upper stem 0–4 inches below rachis	Lower stem 0–2 inches above crown	Upper stem 0–4 inches below rachis
	per cent	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
18131	None	22.0	695.0	1,242.0	973.0	46.0	76.0
18322	None	25.0	532.0	.....	.....	30.0	46.0
18323	0.4	164.0	813.0	.....	.....	Trace	46.0
18327	1.4	50.0	813.0	.....	.....	.....	.....
18321	1.5	1,149.0	1,149.0	.....	.....	1,110.0	276.0
18324	1.6	32.0	709.0	.....	.....	.....	.....
18325	1.8	164.0	813.0	.....	.....	.....	.....
18319	3.1	25.0	668.0	.....	.....	30.0	46.0
18314	11.2	27.0	641.0	.....	.....	30.0	107.0
18315	13.3	61.0	627.0	.....	.....	41.0	107.0
18318	48.3	25.0	586.0	.....	.....	30.0	76.0
18310	53.5	27.0	709.0	46.0	844.0	22.0	76.0
18316	69.0	22.0	371.0	1,352.0	1,023.0	25.0	46.0
18312	77.0	22.0	411.0	682.0	813.0	42.0	46.0
18317	78.0	15.0	166.0	.....	.....	Trace	37.0
18320	114.0	25.0	767.0	.....	.....	Trace	41.0
18311	115.0	27.0	133.0	219.0	813.0	46.0	46.0

* Heading stage, plants 44 days old.

† Ripening stage, plants 66 days old.

It has often been shown that limitation of growth or metabolism by any one of various factors may cause an increase or decrease of various constituents within the plant. The writer has found that the leaves and woody stems of citrus affected with "mottle leaf" contain inorganic phosphate greatly in excess of that in corresponding healthy leaves and branches. It is reasonably certain, however, that this accumulation of inorganic phosphate is not the primary cause of mottle leaf but rather the result of arrested growth and disturbed metabolism.

Since inorganic phosphate may accumulate in plants as a result of nitrogen deficiency, certain physiological diseases, and probably other adverse conditions affecting growth or metabolism, it is apparent that an important limitation is imposed on the use of this method in the diagnosis of phosphate deficiencies. In other words, it will probably be impossible to determine from a test made under sub-optimum conditions with respect to one or more other growth factors whether or not supplemental phosphate will be required when these sub-optimum factors have been corrected. All that may be reasonably hoped for is that the method may indicate *under the particular complex of conditions which actually prevail* whether or not the plant is securing adequate

TABLE 8

Effect of nitrogen deficiency upon the accumulation of inorganic phosphate in green oat plants

SOIL NUMBER	VEGETATIVE STAGE				HEADING STAGE			
	Response to nitrogen	Response to phosphate	Inorganic PO_4 in lower stems		Response to nitrogen	Response to phosphate	Inorganic PO_4 in lower stems	
			Without N	With N			Without N	With N
			p.p.m.	*p.p.m.*			*p.p.m.*	*p.p.m.*
18325	Strong	None	829.0	300.0	.....	.....	.....	.....
18327	Medium	None	1,149.0	334.0	.....	.....	.....	.....
18324	Medium	None	940.0	219.0	.....	.....	.....	.....
18323	Medium	None	709.0	129.0	.....	.....	.....	.....
18313	Medium	None	712.0	368.0	Strong +	None	1,242.0	22.0
18314	Slight	None	528.0	165.0	.....	.....	.....	.....
18315	Slight	None	116.0	64.0	.....	.....	.....	.....
18321	Very slight	None	1,056.0	722.0	.....	.....	.....	.....
18312	Slight	Slight	41.0	23.0	Strong	Medium	259.0	22.0
18320	Slight	Medium	46.0	46.0	Strong	Strong	57.0	25.0
18318	Slight	Slight	46.0	46.0	Medium	Slight +	167.0	25.0
18310	None	Slight	23.0	23.0	Medium +	Medium +	151.0	27.0
18316	None	Slight	35.0	53.0	Slight +	Medium	169.0	22.0
18311	Slight	Medium	41.0	23.0	Slight	Strong	46.0	27.0
18317	Very slight	Medium	46.0	46.0	Slight	Strong	45.0	15.0

phosphate. However, any method that will give accurate results even under this condition will obviously prove exceedingly useful.

Results with lemon plants

Some preliminary data obtained with Lisbon lemon cuttings grown for several months in complete and in phosphate-deficient culture solutions[3] are in harmony with those on the young oat plants. The amounts of inorganic phosphate found in various parts of the citrus plants, grown under the conditions mentioned, are shown in table 9. Except in the young immature leaves, no part of the lemon plants that had been grown from 4 to 7 months in

[3] These lemon cuttings were furnished through the kindness of Dr. A. R. C. Haas.

phosphate-deficient solutions contained more than a trace of inorganic phosphate, and even in the very young developing terminal leaves the amount found was too low to be determined by the method used. These small immature leaves, however, eventually develop into full-sized mature leaves; this is accomplished presumably at the expense of phosphate derived from older parts of the plant. This observation suggests that for leaf growth in the lemon only a very small quantity of inorganic phosphate is needed at any one time provided the supply is adequately maintained.

TABLE 9

Inorganic PO_4 content of lemon cuttings grown in culture solutions

CULTURE SOLUTION	PART OF PLANT	INORGANIC PO_4 OF GREEN TISSUE
		p.p.m.
Complete	Terminal leaf (immature)	133.0
	5th leaf from terminal (immature)	133.0
	10th leaf from terminal (mature)	104.0
	Original leaf of cutting	133.0
	Woody tissue from original cutting	735.0
Moderately deficient in phosphate	Terminal leaf (immature)	Low
	5th leaf from terminal	Trace
	9th leaf from terminal	Trace
	Original leaf from cutting	Low
(Plants grown 4 months)	Woody tissue from original cutting	Trace
Extremely deficient in phosphate	Terminal leaf (immature)	Low
	4th leaf from terminal	Trace
	8th leaf from terminal	None detected
	Original leaf from cutting	Trace
(Plants grown 7 months)	Terminal stem	Low
	Stem 10 inches above base	Trace
	Stem 1 inch above base	Trace
	Stem original cutting	Trace
	Old roots	Trace

In contrast to the low concentration of inorganic phosphate found in the plants grown in phosphate-deficient solutions, all parts of the cuttings grown in complete nutrient solution were relatively high in inorganic phosphate. The woody conductive tissue was especially high.

DISCUSSION

The data obtained with oats clearly indicate that at certain growth stages there is a striking relationship between the inorganic phosphate of green plant

tissue and the nutrient supply. At later growth stages this relationship is less apparent; translocation and transformation changes coupled, perhaps, with a less rapid rate of absorption are probably the factors chiefly responsible. Although the concentration of inorganic phosphate in different parts of the same plant is variable, being highest usually in conductive, embryonic, and storage tissues, all parts appear to be consistent at early growth stages in reflecting the variable supply in the nutrient medium.

It has been shown, however, that if growth is more limited by some other factor than by phosphate, inorganic phosphate may accumulate within the plant. Hence, it will probably be impossible to predict from a test on a given plant grown under sub-optimum conditions with respect to other growth factors whether the phosphate supply would be ample when these other sub-optimum conditions have been corrected. All that may reasonably be expected is that the plant will indicate, *under the particular complex of conditions in which it is growing*, whether or not the phosphate supply is ample.

SUMMARY

Tests for inorganic phosphate in oat plants grown in a series of soils, and in citrus plants grown in culture solutions, have shown the following:

In the vegetative stages, oats limited in growth by insufficient phosphate were exceedingly low in inorganic phosphate; this was true of all parts of the plant. Where not limited by phosphate much higher quantities were found in the green plant. This same condition was found in Lisbon lemon cuttings grown in culture solutions.

Applications of phosphate fertilizer greatly increased the absorption of phosphate by oat plants; all parts of the plant showed this effect in the vegetative stages, the embryonic, conductive, and storage tissues being most markedly affected.

As a result of translocation and transformation changes within the oat plant at later growth stages, the aforementioned relations were not nearly so pronounced. Less rapid absorption of phosphate at these stages may also have accounted in part for the lack of correlation.

Nitrogen deficiencies caused inorganic phosphate to accumulate within the oat plant, even in soils which were deficient in phosphate.

Marked accumulations of inorganic phosphate have been found in the leaves and stems of citrus branches affected with the physiological disease known as "mottle leaf." It is probable that physiological disturbances produced by various other conditions may also cause inorganic phosphate to accumulate within the plant.

Although subject to the limitations noted in the foregoing, the method, if worked out in sufficient detail for different plants, may prove very useful.

REFERENCES

(1) CHAPMAN, H. D. 1931 Notes on use of glass color standards for determination of phosphorus by Deniges colorimetric method. *Indus. Engin. Chem. Analyt. Ed.* 3: 282–284.

(2) CHAPMAN, H. D. 1932 Studies on the blue colorimetric method for the determination of phosphorus. *Soil Sci.* 33: 125–134.

(3) EMMERT, E. M. 1930 A method for the rapid determination of phosphate in fresh plant tissues. *Plant Physiol.* 5: 413–417.

(4) EMMERT, E. M. 1931 The effect of soil reaction on the growth of tomatoes and lettuce and on the nitrogen, phosphorus, and manganese content of the soil and plant. Ky. Agr. Exp. Sta. Bul. 314.

(5) GILBERT, BASIL E., AND HARDIN, LEO J. 1927 The current mineral nutrient content of the plant solution as a possible means of chemical control of optimum fertilization. *Jour. Agr. Res.* 35: 185–192.

(6) GILBERT, B. E., MCLEAN, F. T., AND ADAMS, W. L. 1927 The current mineral content of the plant solution as an index of metabolic limiting conditions. *Plant Physiol.* 2: 139–151.

(7) MCCOOL, M. M. 1926 Relation of soil to plant cell sap. Mich. Agr. Exp. Sta. Quart. Bul. 9: 60–64.

(8) MCCOOL, M. M., AND WELDON, M. D. 1928 The effect of soil type and fertilization on the composition of the expressed sap of plants. *Jour. Amer. Soc. Agron.* 20: 778–792.

(9) PETTINGER, N. A. 1931 The expressed sap of corn plants as an indication of nutrient needs. *Jour. Agr. Res.* 43: 95–119.

(10) PIERRE, W. H., AND POHLMAN, G. G. 1933 Preliminary studies of the exuded plant sap and the relation between the composition of the sap and the soil solution. *Jour. Amer. Soc. Agron.* 25: 144–160.

(11) PLIMMER, ROBERT HENRY ADERS 1913 The hydrolysis of organic phosphorus compounds by dilute acid and by dilute alkali. *Biochem. Jour.* 7: 72–80.

(12) POHLMAN, G. G., AND PIERRE, W. H. 1933 The phosphorus concentration of the exuded sap of corn as a measure of the available phosphorus in the soil. *Jour. Amer. Soc. Agron.* 25: 160–170.

(13) THORNTON, S. F. 1932 A field and laboratory test on plant material for diagnosing phosphorus deficiencies. Ind. Agr. Exp. Sta. Bul. 355.

CARBOHYDRATE SUPPLY AS A PRIMARY FACTOR IN LEGUME SYMBIOSIS

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The necessity for a supply of carbohydrate in legume symbiosis has long been recognized, but the more recent experimental evidence in this and closely related fields has added greatly to our knowledge of the subject. An attempt is made here to present and correlate some of the more pertinent facts, gathered from the literature and from studies carried out in this laboratory, with the view to showing in greater detail the manner in which the bacteria and host respond to varying rates of photosynthesis.

These studies seem to show that the available carbohydrate supply (chiefly sugars and starch) is a primary factor in determining nodule location, growth, and size; harmful effect of fixed nitrogen on nodulation; quantity of nitrogen fixed by good strains; disintegration of nodules; and other similar phenomena. It is desired especially to emphasize that this discussion, unless otherwise stated, deals with plants growing under *normal soil conditions where nitrogen is rather deficient.* Certainly under many other conditions, such as high acidity, deficient calcium, and insufficient moisture [*see* excellent monograph by Fred Baldwin, and McCoy (14)], other factors may outweigh carbohydrate supply in importance. It should also be stressed that this paper deals primarily with symbiosis involving *good nitrogen-fixing bacterial strains*, and is not concerned with the reasons for variations in infectiveness (ability to produce nodules) or effectiveness (ability to supply the host plant with nitrogen) of different strains. Very little is known about the reasons for these variations, and whether the carbohydrate supply is a factor of major importance remains to be determined.

CARBOHYDRATE SUPPLY AND NODULE FORMATION

A supply of carbohydrate is needed for every step in nodule formation, but the quantity required varies in the different stages of development.

Traces of organic matter are normally liberated from the roots of plants in the forms of sloughed-off root cells and possibly also as excretions. It is

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probable that this food supply stimulates the growth of legume bacteria present. A high carbohydrate concentration in the root is not a prerequisite for bacterial entrance.

Why do nodule bacteria enter roots? It has been shown that they will enter and form nodules on roots growing in (*a*) dilute mineral solutions, (*b*) sugar solutions, (*c*) weak nitrate solutions, and (*d*) complete nutrient media. This indicates that they do not penetrate roots for any very specific reason. They seem to grow wherever the growth conditions and food supply are satisfactory, whether outside or inside the host cell.

Several interesting phases of bacterial entrance, not yet worked out in complete detail, have been considered by McCoy (31) and by Thornton (53). The nature of the infection thread is described fully by Thornton (52) and the development of the nodule by Thornton (53) and by Brenchley and Thornton (7).

It is at the time of the growth of nodule tissue that the carbohydrate supply becomes of major importance. The penetration of the infection thread stimulates rapid cell division and growth; likewise, there occurs a great increase in bacterial numbers. Very shortly thereafter nitrogen fixation begins, and a marked growth of the host tissue follows. All of the activities of the plant are suddenly accelerated and these require an ever increasing supply of carbohydrate.

It should be borne in mind that the sugar synthesized in the leaves, moves to other parts of the plant by a process somewhat similar to, but more rapid than, diffusion. However, during active photosynthesis, it commonly accumulates to a considerable extent in the tops, as shown by Mason and Maskell (32) in the cotton plant. Miller (33) mentions several experiments with both legumes and nonlegumes showing that there is an osmotic pressure gradient in plants. This gradient is undoubtedly a factor of considerable importance in legume nodulation.

CARBOHYDRATE UTILIZATION AS A FUNCTION OF AMOUNT SYNTHESIZED

Since good nodule development depends upon an abundant carbohydrate supply, it is proposed to show what use the plant makes of the photosynthetic carbohydrate as the amount formed in the leaves varies from very little to maximum. The facts are shown diagrammatically in figure 1.

This figure is a composite picture for various species of legumes, drawn from bits of information gathered chiefly from the many articles referred to in this paper. It shows the manner in which the carbohydrates are utilized by legumes, growing in an inoculated nitrogen-deficient soil under conditions such that the rate of photosynthesis varies. As is well known, many factors, particularly light intensity, carbon dioxide supply, chlorophyll content, and temperature, greatly affect carbohydrate synthesis. Data showing the weights of the tops and roots of various legumes grown under widely different conditions are rather abundant; similar data giving nodule weights are less abundant but adequate for the present purpose. The respiration data, considered quanti-

tatively, are the least satisfactory, but qualitatively they bear out the ideas conveyed in figure 1. Emphasis should not be placed upon the details of the curves but rather upon the broad, general relationships of amount of carbohydrate synthesized in the leaf to respiration, growth, and nodulation.

Before the figure is discussed in detail it should be emphasized that during the active growth of a plant a fairly wide variation in the rate of photosynthesis does not necessarily produce a corresponding wide variation in the concentra-

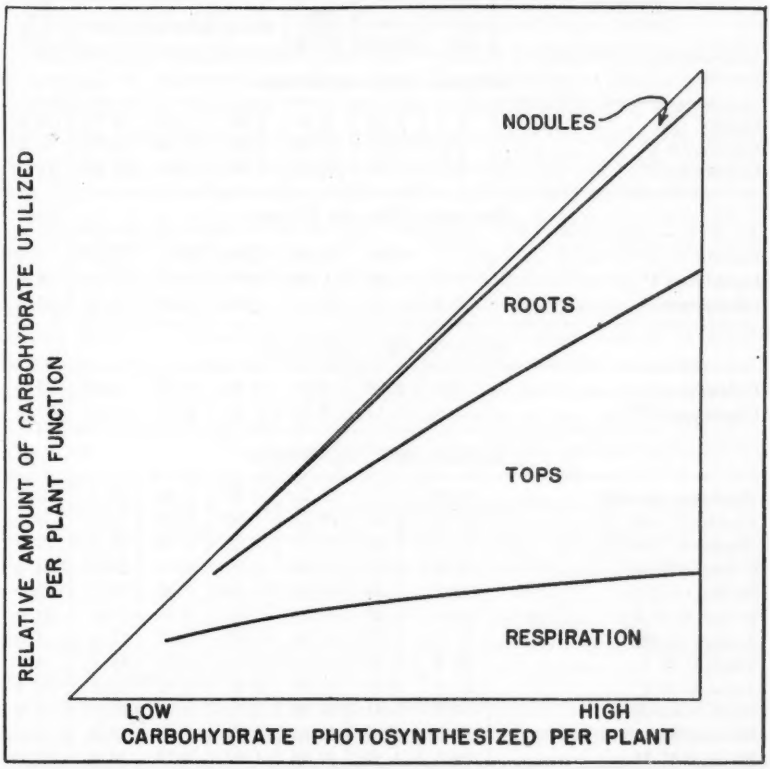

FIG. 1. CARBOHYDRATE UTILIZATION IN LEGUMES AS A FUNCTION OF AMOUNT SYNTHESIZED

tion of soluble carbohydrates in the plant juice, assuming that conditions for growth are satisfactory (3). The increased sugar, above the needs for respiration, is used in the growth of new plant tissues, or is stored largely as starch. The data of Arthur, Guthrie, and Newell (5), a small portion of which is shown in table 1, well illustrate this point. Although the sugars, total available carbohydrate, and the carbohydrate-nitrogen ratio usually increased with increased light and carbon dioxide, it was the dry weight of the plant which showed the greatest increases.

Where photosynthesis is proceeding at a very slow rate it will be noted from figure 1 that the carbohydrate is consumed largely or wholly in respiration.

TABLE 1

Chemical composition of the aerial portion of legumes grown under different environmental conditions

TREATMENT	WEIGHT PER PLANT	TOTAL NITROGEN	CARBOHYDRATE—PER CENT DRY WEIGHT				CARBOHYDRATE / NITROGEN
			Acid hydrolyzable	Sucrose	Dextrose	Total	
	gm.	*per cent*					
Red clover, 1925. Age 76 days							
Control	1.1	3.44	9.61	2.28	1.86	13.75	4.0
Greenhouse 1*	6.0	2.73	11.80	2.50	2.66	16.96	6.4
Greenhouse 2*	9.2	1.96	16.36	2.99	2.70	22.05	11.2
Red clover, 1926. Age 71 days							
Control	2.1	3.18	12.25	2.04	3.52	17.81	5.6
Greenhouse 1*	2.2	2.88	11.49	1.93	4.12	17.54	6.0
Greenhouse 2*	3.9	1.76	19.14	2.30	5.84	27.28	15.6
Red clover, 1927. Age 66 days							
Control	0.6	2.90	4.92	2.30	1.44	8.66	3.0
Greenhouse 2*	5.9	2.13	15.53	4.86	4.58	24.97	11.6
Soybeans, 1925. Age 40 days							
Mandarin, control†	7.8	3.71	19.22	2.06	1.38	22.7	6.1
Mandarin, G.H. 1†	35.8	2.97	19.33	2.00	2.69	24.0	8.1
Mandarin, G.H. 2†	32.1	1.81	26.12	1.81	3.36	31.3	17.2
Peking, control†	3.8	4.12	15.03	2.06	0.76	17.9	4.4
Peking, G.H. 1	19.5	3.32	15.97	2.10	1.38	19.5	5.9
Peking, G.H. 2	38.1	2.19	19.54	2.35	3.59	25.4	11.6
Tokio, control†	11.6	3.73	13.63	1.66	0.81	17.1	4.6
Tokio, G.H. 1	30.8	3.79	15.22	2.37	0.68	18.3	4.9
Tokio, G.H. 2	52.1	2.57	16.75	2.57	3.92	23.2	9.1
Biloxi, control†	14.2	3.81	16.40	1.72	0.91	19.0	5.0
Biloxi, G.H. 1	52.8	3.19	14.86	2.54	1.63	19.0	6.0
Biloxi, G.H. 2†	50.8	1.50	30.84	1.82	2.28	34.9	23.0

Control plants grown in ordinary greenhouse.
Greenhouse 1, greenhouse plus 6 hours artificial light daily.
Greenhouse 2, same as greenhouse 1 plus extra CO_2 (about 0.3 per cent).
Greenhouse 2 (1927), received 12 hours artificial light and extra CO_2.
* Flowering.
† Fruiting or flowering.

With increased photosynthesis a point is soon reached where the supply of carbohydrate is adequate for appreciable growth. Usually considerably more of the carbohydrate is used in the production of leaves and stems than of roots,

but the relative amounts used in the two portions of the plant vary widely with the plant species and growth conditions. No appreciable nodule development takes place until the carbohydrate supply is adequate for a fair amount of root growth, as has been shown experimentally many times (3). It is obvious, then, that practically any set of conditions unfavorable for root growth is at the same time more unfavorable for nodulation.

If the carbohydrate supply is adequate for good nodule formation it will be observed from the figure that additional carbohydrate merely means more tops, roots, and nodules, the increases of each being somewhat regular.

Respiration, which consumes practically all of the carbohydrate where little is synthesized, consumes a smaller and smaller percentage of the total where photosynthesis is increased. The absolute amount consumed in respiration under these conditions constantly increases, of course, because of increased dry matter and somewhat higher sugar concentration, but relatively on a per plant basis a smaller percentage of the total carbohydrate is so burned up.

The rate of photosynthesis at which appreciable nodule growth will occur varies with the legume species and other factors but particularly with the quantity of soluble soil nitrogen. If the supply is very abundant a large percentage of the carbohydrate not required for respiration will go into top growth, leaving little for root growth, even though the rate of photosynthesis is rather high. If, on the other hand, the supply of fixed nitrogen is limited, as specified in connection with figure 1, an abundance of top growth without considerable root growth and fair nodulation is impossible for the obvious reason that nodules must form and nitrogen fixation begin before there is an adequate supply of nitrogen for either top or root growth.

The results of Eaton (12) with soybeans well illustrate an intermediate condition, so far as nitrogen is concerned. Even though a nutrient solution containing small amounts of nitrate nitrogen was added daily, he observed that growth and nodule development were correlated with the length of day, severity of leaf clipping, and percentage of carbohydrates. Certain other correlations would, undoubtedly, have been in evidence if a nitrogen-free medium had been used.

Nodule growth normally lags slightly behind root growth probably partly because of the difficulty of getting sufficient carbohydrate into the nodule rapidly enough. Not only is the base of a nodule usually constricted, but the quantity of carbohydrate consumed per unit of nodule tissue is probably higher than per unit of root tissue.

CARBOHYDRATE ABSORPTION THROUGH THE ROOT

Although emphasis in the foregoing discussion has been placed upon photosynthetic carbohydrate, the fact should not be overlooked that plants can to some extent absorb certain sugars through the roots and utilize them in the same manner as if synthesized in the leaves (33, p. 239–241). If sugar is absorbed in this manner by plants growing in normal light, then both sources of

sugar will be used simultaneously and, since the normal sugar gradient will no longer exist, we should expect an unusually good growth of roots and with little lag in nodule development. The marked increase in root growth following the addition of sugars has been observed frequently. The data of Knudson (23), a portion of which is given in table 2, illustrate this point. Vetch plants, growing in the presence of sucrose, showed a top-root ratio of 1.2 as contrasted with a ratio of 4.6 without sugar.

Several experiments have been reported which show the effect of sugar additions on nodules, for example, Golding (18), Prucha (42), Wilson (57), Leonard (26), and Georgi, Orcutt, and Wilson (16). Wilson (58) and Lewis and McCoy (27) obtained nodules on plant roots kept in sugar solutions in the dark. Hopkins and Fred (21) observed that mannitol partially counteracted the effect of nitrogen additions on nodule production, and Allison and Ludwig (3) obtained a similar effect with sucrose.

TABLE 2

Influence of various sugars on the growth of vetch in water cultures under sterile conditions

(Duration of experiment 39 days)

CULTURE SOLUTION	NUMBER OF PLANTS	DRY WEIGHT OF ROOTS	DRY WEIGHT OF TOPS	TOTAL DRY WEIGHT	AVERAGE TOTAL DRY WEIGHT PER PLANT	GAIN IN WEIGHT PER PLANT*	SUGAR ABSORBED	TOP/ROOT RATIOS
		mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	
Check (no sugar)	2	13	67	80	40.0	11.0		5.2
Check (no sugar)	2	15	68	83	41.5	12.5		4.5
Check (no sugar)	1	8	32	40	40.0	11.0		4.0
Lactose	1	16	40	56	56.0	27.0	49.0	2.5
Glucose	2	41	81	122	61.0	32.0	72.6	2.0
Glucose	1	30	62	92	92.0	63.0	66.5	2.1
Maltose	1	20	42	62	62.0	33.0	24.4	2.1
Fructose	2	39	75	114	57.0	28.0	113.0	1.9
Sucrose	1	55	60	115	115.0	86.0	133.4	1.1
Sucrose	2	77	96	173	86.5	57.5	141.0	1.2

* The weight of each seed was 29 mgm.

RELATIVE CARBOHYDRATE REQUIREMENTS OF THE HOST PLANT AND OF THE BACTERIA

A better understanding of the rôle of carbohydrate in legume symbiosis may be had if consideration is given to the relative needs of the bacteria and host for the carbohydrate synthesized in the leaves. In order to produce 1 gm. of dry weight about 5 to 20 gm. of glucose are required in the case of most aerobic bacteria, whereas only about 1.35 gm. are required for the more efficient higher plants (29, 50, 54).

What are the relative weights of the bacteria in the nodule and of the host

plant? The dry weight of nodules on annuals grown under very exceptionally favorable conditions is about 7 per cent of the total dry weight of the whole plant, as shown by Giöbel (17), and by Jones and Tisdale (22). Probably less than 1 per cent of the total weight of the average legume plant represents bacterial cells.

The activity of the bacteria in the nodule is an important consideration. The evidence shows that they are present largely as bacterioids, which are not being digested rapidly (17), and which are mostly incapable of multiplication, but which are almost certainly consuming some energy in respiration. Furthermore, the energy requirements for nitrogen fixation, as is pointed in the following, are probably negligible.

These facts indicate very strongly that the bacteria in the nodules consume a very small proportion of the total carbohydrate photosynthesized.

There is another type of evidence that throws light on the carbohydrate requirement of the host, relative to that of the nodule bacteria. Newton (37) and Reinau (43, 44, 45) have shown that the roots of inoculated legumes give off about twice as much CO_2 as do the roots of nonlegumes. Reinau does not, however, prove satisfactorily that this difference is due to the respiration of the nodule bacteria rather than to the natural differences in the plant species, wholly apart from the bacteria.

Let us, however, assume that (*a*) the excess respiration is due to the bacteria as Reinau thought, (*b*) unit weights of root and top tissues (exclusive of bacteroidal tissue) respire at the same rate, (*c*) the legume roots represent 30 per cent of the total weight of the plant, and (*d*) legumes use 25 per cent of the photosynthesized carbohydrate in respiration. Under these conditions about 5.8 per cent of the carbohydrate synthesized is used by the nodule bacteria in respiration and nitrogen fixation. According to Lundegårdh (29) the respiration rate of the tops is usually higher than that of the roots. The recently reported data of Müller (36) show that *Sinapis alba* had relative respiration rates of 2.95 and 1.36 for the tops and roots. If we use these figures in the foregoing calculations then only 3.5 per cent of the carbohydrate photosynthesized is used by the bacteria.

Both lines of evidence presented indicate, then, that the bacterial requirements for respiration and nitrogen fixation are probably not greater than 3 to 6 per cent of the total carbohydrate photosynthesized under good growing conditions. This estimate is, admittedly, not very accurate, and is probably high rather than low.

CARBOHYDRATE AND THE LOCATION OF NODULES

Under most conditions nodules are located principally at the tops of the root system on or near the main roots, especially in the case of annuals or during the first year's growth of other legumes. Several reasons have been advanced for this, such as better aeration and immunity. Evidence is presented in the following which seems to show that the carbohydrate supply is a primary

factor in nodule location, assuming uniform inoculation at the time of seeding, and a satisfactory soil environment. Under many conditions, of course, carbohydrate supply would be a secondary factor.

Normal nodules. The experiments of Nobbe, Schmid, Hiltner, and Hotter (39) and of Otis (40) showed that where plants were inoculated at different depths the nodules always formed only at the point of inoculation. Löhnis (28) and Ludwig at this laboratory observed that delayed inoculation resulted in nodules occurring much lower on the root system. Normally not only are most of the nodules located near the soil surface but also near the tap root, whereas other rootlets located just as near the surface but several inches from the larger central roots show few or no nodules. Aeration is apparently a very secondary factor under these conditions. The writer has frequently observed that in the case of soybean seedlings growing in the presence of a heavy inoculation, a single rootlet may have 10 to 20 nodules. At first they are of uniform size, but soon the ones nearest the main root become much larger while those farther away remain small or disappear. The whole tip of the rootlet beyond the nodules frequently appears stunted, almost certainly as a result of under-nourishment.

Nodule development is usually best in the region of the tap root and nearest the source of carbohydrate supply. Löhnis (28) observed that the upper portions of the root system commonly show starch deposits, while the lower roots, especially the root tips, are free from these. Uninoculated plants, however, if growing in a nitrogen-deficient medium, usually show starch deposits in most of the root cells except at the extreme tip. After nodules have formed on the upper roots and are actively fixing nitrogen the abundant supply of nitrogen, originating in the nodule, markedly stimulates growth and utilization of the carbohydrate as fast as formed.

In the experiments already noted, where inoculation was delayed, sufficient carbohydrate was present in the lower portions of the root system to enable nodules to develop. Active photosynthesis and slow growth, due to inadequate nitrogen, must necessarily result in carbohydrate accumulation. The failure of nodules to form on the upper portion of the root system, already well-nodulated at lower depths, may be due either to insufficient carbohydrate or to lack of root hairs.

Nodules in water cultures. Nobbe and Hiltner (38), Hiltner (19), and the writer observed that legumes produced small, inefficient, scattered nodules when grown in nitrogen-free nutrient solutions. When some of the solution was poured off, a strikingly rapid growth took place in the nodules located above the solution, and plant growth increased markedly.

We may, of course, explain this by simply saying that nodules develop in air better than they do in water, but in the opinion of the writer a more plausible and fundamental explanation is one based on the carbohydrate-nitrogen relationships. In the submerged nodules no appreciable quantity of nitrogen was fixed, but photosynthetic conditions were favorable, and nodules could form

everywhere. When a portion of the solution was poured off, a mass of large, normal nodules, active in nitrogen fixation, formed above the liquid, and the carbohydrate supply was then used largely to meet the increased plant and bacterial needs. Even as early as 1891 Laurent (25) observed that pea nodules grown submerged in water cultures contained much starch and few bacteroids and fixed little nitrogen. Nodules formed above the water contained little starch, were packed with bacteroids, and fixed considerable nitrogen.

The writer grew soybeans in a 0.5 per cent sucrose-mineral medium, containing no nitrogen. About 40 small scattered nodules were formed per plant both in the presence and in the absence of sugar. Apparently little or no nitrogen was fixed. In this case the plants grown both with and without sugar were high in carbohydrate, and hence, as expected, added sugar had no effect.

Non-effective nodules. The results of several recent workers have agreed in showing that nodules produced by poor nitrogen fixing strains of bacteria are usually small, rather numerous, and widely scattered over the root system. With ineffective strains the carbohydrate-nitrogen ratio is necessarily wide, starch occurs (28) almost to the root tips, and the nodules occur all over the root system. The reason for the failure of the ineffective nodules to become large or to form in excessively large numbers is not known. The explanation may revolve in part about the strain, but it seems more probable that nitrogen deficiency is the more important factor.

It has recently been shown by Dunham and Baldwin (11) that where plants are first inoculated with a non-effective strain and become well nodulated, additional nodules will form if an effective strain is then added. If the plants are first inoculated with good strains and several days later with poor strains the nodules are due wholly, or nearly so, to the good bacteria. A simultaneous inoculation with good and poor strains results in nodule formation by both. These workers point out that both good and poor strains vary in their ability to inoculate a plant, but one group is not necessarily superior to the other in infectiveness. Any given strain may be made either more or less effective in nitrogen fixation, according to Allen and Baldwin (2) and others, by repeated plant passage.

These findings agree with the idea that nodule formation is closely connected with carbohydrate supply in the root if we make the logical assumption that with ineffective strains only present, the carbohydrate is more plentiful than if effective strains are present. More information is needed, however, before a positive conclusion can be reached.

Nodules growing in a nitrogen-free atmosphere. The results of Kossowitsch (24) and of Whiting (56), particularly the latter, are of unusual significance in the present discussion. In the experiments of Kossowitsch, inoculated pea seedlings placed in a sand medium with the roots in a mixture of oxygen and hydrogen made a negligible growth but formed numerous nodules. Whiting grew inoculated cowpea seedlings with the roots in a gas mixture consisting of 97 per cent oxygen and 3 per cent carbon dioxide. The plants made little

growth but formed many very small nodules scattered over the root system practically to the root tips. Where nitrogen gas was present good fixation occurred and the nodules in this case were very large and located mostly on the upper and older part of the root system.

In these experiments, where the roots received neither free nor fixed nitrogen, the nodules formed were almost identical in appearance and location with those produced by non-effective bacterial strains on plants growing in air. This definitely eliminates the bacterial strain as the sole cause of the location and size of the ineffective type of nodule. Under the experimental conditions of Kossowitsch and of Whiting, as well as in the presence of non-fixing strains, an abundance of carbohydrate accumulates in the roots, and nodules can form all over the root system but they remain small, probably because of insufficient combined nitrogen.

It would seem from the foregoing experiments that nitrogen fixation is somewhat incidental in legume symbiosis, since nodulation occurred even though nitrogen fixation could not take place.

Nodules growing on plants supplied with increased carbon dioxide. Riedels (46) reported that the use of additional CO_2 increased the number of nodules on beans 5-fold and increased plant growth. Wilson, Fred, and Salmon (59) commonly obtained increases of 100 to 200 per cent in dry weights and nitrogen fixation with red clover when the partial pressure of CO_2 was increased from that in normal air to 0.2 or 0.4 per cent. The plants had two or three times as many nodules as the controls in air, and the size was greatly increased. Where the plants were grown in 0.5 per cent CO_2 the nodule distribution was very similar to that of plants inoculated with poor strains of the organisms. The largest nodules were clustered near the crown on the tap root with the small, round variety scattered among the secondary roots.

These data emphasize the important rôle that carbohydrate supply plays in nodule location. Under conditions of increased CO_2 the rate of photosynthesis was so rapid that even though the nodules were actively fixing nitrogen there was adequate carbohydrate to allow small nodules to form on the secondary roots, just as occurs with non-effective strains where carbohydrates accumulate because of lack of nitrogen.

CARBOHYDRATE SUPPLY AND THE PHYSIOLOGICAL CONDITION OF THE NODULE

The condition of the nodule is also dependent upon the carbohydrate supply. An attempt is made in the following to present the evidence for this statement, and in so doing special reference is made to certain instances of abnormal nodules.

Normal nodules. A description of the nodule from broad bean (*Vicia faba*), as reported by Brenchley and Thornton (7), may be considered as rather typical. Usually some starch is present, especially near the base of the nodule, but not in excessive amounts. Very starchy nodules are likely to be rather ineffective and, of course, contain fewer bacteria, as Peirce (41) pointed out

many years ago. In normal nodules little or no starch is found in the apex of the nodule where the meristem tissue is located. The proper functioning of the nodule seems to demand that a readily available carbohydrate supply be maintained.

Bean nodules. Bean nodules are of interest because they commonly, or at least frequently, fix little or no nitrogen. They would, therefore, if the ideas presented here are correct, be expected to contain an abundance of starch under conditions of normal photosynthesis.

Deposition of starch, according to McCoy (30), begins early in the development of these nodules, starch grains being recognizable in a section of an infection spot so young as to have spread only part way across the root cortex. She states that there is decidedly more starch in the average bean nodule than in the normal nodules of other plants. McCoy was of the opinion that starch grains once formed are inaccessible to the bacteria so long as they are surrounded by the bodies of the plastids. If this is the case then the condition in

TABLE 3

The effect of darkening or removing tops on the starch content of young pea nodules

	STARCH REACTION AFTER 3 DAYS—PER CENT				
	0	Trace	Weak	Medium	Strong
Group A—Plants in light	0	0	16	40	44
Group B—Plants darkened	7	36	37	18	2
Group C—Plants with tops removed	12	10	31	40	7
	STARCH REACTION AFTER 5 DAYS—PER CENT				
Group A—Plants in light	0	2	14	34	50
Group B—Plants darkened	19	55	16	5	3
Group C—Plants with tops removed	3	31	38	28	0

these nodules must be somewhat abnormal or pathological. In nodules on other species of legumes the starch is quickly used by the host and bacteria when the plants are darkened or other methods used to cut off photosynthesis. The results of Rippel and Poschenrieder (47), summarized in table 3, illustrate this point. According to Löhnis (28) neither effective nor ineffective strains of nodule bacteria can utilize starch. The cells of higher plants can, however, do this readily (33) under normal conditions.

Boron-deficient nodules. The necessity for an adequate supply of carbohydrate is well-illustrated in the case of broad beans grown in the absence of boron. Brenchley and Thornton (7) observed that in such plants the early development of the nodule is normal whereas the later development depends largely upon the extent to which vascular strands are formed. In the absence of boron frequently no strands develop.

In these boron-deficient nodules the infection thread, containing rod forms, undergoes a remarkable development and finally breaks up the nodule tissue.

The bacteria usually remain as rods and cocci and may become actively parasitic and destroy the protoplasm of the host cell early in the life of the nodule. Where there is a partial development of vascular strands there is a partial development of bacteroidal tissue and the behavior of the bacteria is intermediate. These investigators logically concluded that the bacteria prefer to utilize the carbohydrate supply furnished by the plant through the vascular strands, but if this is inadequate they may under certain conditions use the protoplasm of the host cell.

Disintegrating nodules. According to Thornton (53) three conditions are known under which a good strain of the nodule organism may become parasitic and destroy the tissues of the host. These are (*a*) where plants such as the broad bean are grown in a boron-deficient medium, (*b*) where the host plant is put in the dark, and (*c*) in old nodules. In all cases the lack of an adequate carbohydrate supply is, apparently, the primary reason for the parasitism. Even as early as 1890 Frank (13) pointed out that starch disappears as the nodule starts to disintegrate.

Disintegration of both old nodules and nodules in the dark, according to Thornton, is started by coccoid rods, usually found in the residual pieces of infection thread. These bacteria multiply and remain as rods as they invade the cell walls, finally causing them to collapse. The conditions are similar in the boron-deficient nodules, as already pointed out. The observations of Milovidov (34) were essentially in agreement.

What prevents the disintegration of normal nodules? Thornton (53), as well as others before him, brought up the question of why bacteria growing in normal nodules do not increase up to the limit set by the total energy supply, consuming both the carbohydrates brought to them and also the host tissues. He, as well as a few earlier workers, suggested that the air supply may be the limiting factor. Recent researches throw new light on this question and indicate that a number of factors may be involved.

The organisms exist in the young infection threads as actively growing rods. The tip and younger portions of the infection thread ordinarily consist of irregular zoogloeal masses of bacteria which are largely released into the host cells. These soon cease to divide and are converted into bacteroid forms, which, according to Müller and Stapp (35), Almon (4), Schaede (49), and others, are incapable of multiplication. The active rod forms are limited (52) largely or wholly to the infection threads and to the region just behind the meristem cap where continuous infection of the new cells takes place. Traces of old infection threads with a few rods inside are commonly found scattered throughout normal bacteroidal tissue. This older infection thread, or sheath, according to Thornton (52) and McCoy (31), is continuous with the cell walls of the host plant. McCoy made the very important observation that there is no sheath around the infection thread as it crosses the middle lamella of the cell wall. This indicates that the sheath is deposited by the plant cell as a defense mechanism against the bacterial invasion.

The facts, then, seem to indicate very strongly that in a normal nodule the bacteria are able to live and multiply normally only if protected from the cell contents by the infection thread, whether it be slime-like in nature or resembling a cell wall. If they are liberated into the host cell and lose their protective covering they are ordinarily soon converted into bacteroids. It is not known whether the environment inside a living cell is normally unfavorable for bacterial growth, whether the host secretes something to inactivate the bacteria, or whether the end products of bacterial action, including carbon dioxide, make the environment unsuitable. At any rate it scarcely seems that the oxygen supply is the primary limiting factor, since the bacteria inside these same nodules may grow rapidly if protected by the young infection thread. Furthermore, Barthel (6) and Allison and Hoover (unpublished data) have shown that legume bacteria make good growths in nitrogen-oxygen mixtures of about 0.50 to 2 per cent oxygen. It is improbable that the oxygen concentration inside the nodule would ordinarily be as low as these percentages.

A study of why the bacteria in a normal nodule do not destroy the host tissue limits itself, then, if the foregoing facts are correct, to a consideration of the behavior of the few active rod forms (capable of reproduction) found there, namely, those protected by the infection thread. The indications are that these bacteria do not ordinarily multiply rapidly and hence need little carbohydrate. If these rod forms do multiply rapidly in the presence of available carbohydrate they normally pass out into the cell and are converted into bacteroids. Such continued rapid bacteroid formation is, of course, impossible for purely physical reasons, if for no other. The host cells are soon filled to capacity and the bacteroids are digested slowly, if at all. The situation is obviously quite different from that in a nodule lacking in carbohydrate where the bacteria attack the middle lamella of the cell wall (53) to get energy and, having broken this down, the whole cell collapses.

Although the observations considered in the foregoing clearly point to the necessity for an adequate supply of carbohydrate in order for a nodule to remain healthy, it must be admitted that the available information is still somewhat limited. Much of the discussion revolves around the idea that the nodule bacteria can under certain conditions attack host tissues. Further studies of this phenomenon in particular are needed.

DECREASED NODULE FORMATION IN THE PRESENCE OF COMBINED NITROGEN

A consideration of legume symbiosis from the carbohydrate standpoint allows us to explain the harmful effect of high concentrations of soluble nitrogenous compounds on nodule formation. This subject is considered at length by Allison and Ludwig (3).

If a plant is grown in the presence of an abundance of fixed nitrogen an unusually large percentage of the carbohydrate synthesized is used for top growth and a proportionately smaller root system necessarily develops (15). Since the conditions are usually unfavorable for good root growth we could not

expect nodules to develop normally under these conditions. With moderate nitrate applications nodule tissue may form, but under the same conditions considerable root tissue also develops, the extent of development of both depending primarily upon the root carbohydrate supply, if other growth conditions are satisfactory. The bacteria normally remain alive and ready to produce nodules whenever the carbohydrate supply is adequate, not adequate primarily for the bacteria but for the growth of the tissue of the higher plant in which the bacteria will later live.

It is well to point out the close similarity, so far as nodule development is concerned, between conditions of (*a*) normal light intensity and high soluble soil nitrogen, and (*b*) low light intensity and normal soil nitrogen. Under both conditions the available root carbohydrate is low, and growth in this region is limited.

CARBOHYDRATE SUPPLY AND NITROGEN FIXATION

A high rate of photosynthesis usually results in a high rate of nitrogen fixation. Plant growth, nodule development, and nitrogen fixation usually closely parallel one another, as many workers (14) have shown. Almost any condition which favors increased plant growth, other than the application of large quantities of nitrogenous fertilizers, also favors increased activity in the nodule and the formation of new nodule tissue.

There is one particularly outstanding case, however, where nitrogen fixation is negligible even when growing conditions are good and effective bacteria are present. Sometimes seedlings fail to start active nitrogen fixation for several days or weeks, even though kept under excellent light conditions and inoculated with good strains. Nodules will form in the usual period but frequently remain small, and the plant shows no benefit from them. Hiltner and Störmer (20), Rüffer (48), Weber (55), as well as others have observed and discussed this hunger condition. Allam (1) and Rüffer (48) found that in the case of soybeans the nitrogen hunger period was not as much in evidence during the fall as during the summer. Several investigators, for example Fred and Graul (15), have mentioned the favorable effect of small amounts of nitrogen in overcoming this condition. The lag period may last as long as 1 or 2 months in extreme cases, after which nitrogen fixation frequently proceeds at an unusually rapid rate (48) accompanied by the growth of considerable new nodule tissue. The exact cause of the hunger condition is not known with certainty; although excess carbohydrate undoubtedly plays an important rôle, other factors seem to be involved.

ENERGY REQUIREMENTS FOR NITROGEN FIXATION

The literature dealing with legume symbiosis is full of statements or implications to the effect that large quantities of energy are consumed by legume bacteria in the process of nitrogen fixation. Such statements have never been proved and are, in fact, very probably not true. The confusion that seems to

exist is probably due largely to a lack of uniformity of viewpoint in defining the term "energy requirements for nitrogen fixation."

Theoretically, from the strictly chemical standpoint, what are these requirements? The answer to this question can not be given exactly because the chemical steps in the process of nitrogen fixation are unknown. The order of magnitude of these requirements is, however, well known. They are, at most, very small, assuming 100 per cent efficiency of the process; in fact, the nitrogen fixation step may be exothermic and yield energy. Burk (8) points out, for example, that it would be possible for nitrogen to react with carbohydrate to form an alpha-amino acid, $C_6H_{12}O_6N_2$, which process is exothermic. If we assume that the free nitrogen is reduced to ammonia, corresponding to a proteinaceous form, then a definite but small amount of energy is required, since the necessary hydrogen must be derived from organic compounds of carbon. The maximum amount of energy necessary in this case under ideal conditions, according to Burk, corresponds to 1.3 gm. of glucose per gram of nitrogen fixed as ammonia or protein.

Burk determined that the energy requirements for nitrogen fixation, apart from growth, are negligible in the case of *Azotobacter*. Although the apparent energy needs were often large, he found that this was due to an unnecessary consumption of carbohydrate's occurring simultaneously with, but independently of, fixation.

Nitrogen fixation in legumes seems to be a comparable case, the energy requirements for fixation sometimes appearing to be large. When such is the case the apparent energy requirements can almost invariably be accounted for on purely physiological grounds that have no connection with the energy consumed in the chemical process itself, apart from growth.

The definition of the term "energy requirements for nitrogen fixation," then, as used here, is that energy consumed only in the chemical process of fixation and excludes that consumed in the respiration and growth of the bacteria and host. If large quantities of energy are needed for fixation it can only be because the efficiency of the chemical process of fixation is extremely low.

The results of Christiansen-Weniger (10) led him to conclude that the energy requirements for fixation are small. These conclusions were based on experiments with legumes grown with free and fixed nitrogen, exposed to different light intensities, with the calculations based upon the differences in dry weights and nitrogen fixed. The maximum energy requirements for fixation in two experiments were not greater than 5.6 and 7.2 gm. dry matter (22.4 and 28.9 Cals.), respectively, per gram of nitrogen fixed. This includes the requirements for bacterial growth and respiration, which he believed were at least as large as these figures. There were other good physiological reasons for believing these values were too high. Although these data are not free from criticism, Christiansen-Weniger was apparently well aware of most of their shortcomings, and his conclusions seem justified.

Recently Allam (1) concluded from experiments somewhat similar to those

of Christiansen-Weniger that the bacteria required 26.0 and 12.6 gm. of dry matter (104.0 and 50.4 Cals.), respectively, to fix 1 gm. of nitrogen in two experiments. Since his experiments were carried out under conditions of such deficient light that growth and nitrogen fixation were abnormal, the data furnish little information as to the energy requirements for chemical fixation apart from respiration and growth.

The following factors, to a large extent uncontrollable, enter into the final results of such experimentation as reported by Christiansen-Weniger and Allam:

(*a*) The amount of nitrogen available for the growth of a plant affects the leaf surface and, in turn, photosynthesis. The higher the nitrogen content, usually the greater is the leaf surface per unit dry weight. For instance, Müller (36) found in studies with *Sinapis alba* that plants receiving adequate nitrogen had a leaf area of 126 sq. cm. per gram of total dry substance while similar plants undergoing nitrogen hunger had a leaf area of only 52.8 sq. cm.

(*b*) It is impossible to keep the rates of growth of plants fed free and fixed nitrogen the same, the latter usually being ahead, which means that the total carbohydrate photosynthesized is greater in this case.

(*c*) Plants given an adequate supply of fixed nitrogen sometimes have a slightly wider top-root ratio than do nodule-fed plants, with a larger proportion of the dry matter in the form of leaves actively building up carbohydrate. With ordinary quantities of nitrogen the effect on the top-root ratio would be small.

(*d*) The carbohydrate level in plants fixing nitrogen is commonly slightly higher than in plants given adequate fixed nitrogen. This is evidenced by the slightly lower percentage of nitrogen (wider carbohydrate-nitrogen ratio) usually found in plants living on atmospheric nitrogen. Nodules do not even form until sufficient carbohydrate has accumulated to allow at least a fair root growth.

(*e*) The amount of nitrogen in a plant affects the chlorophyll content of the leaves, which, in turn, affects the rate of photosynthesis somewhat. Any tendency toward a hunger condition in nodule-bearing plants would ordinarily result in a slightly lower chlorophyll content. Müller (36) observed that the assimilating intensity of leaves from plants receiving insufficient nitrogen was about half as great as from those supplied with plenty of nitrogen under conditions of abundant light; as the light intensity decreased the differences also decreased rather uniformly.

(*f*) The nodule bacteria require energy for respiration, apart from any that may be needed for fixation, and it is difficult to separate the two requirements experimentally.

(*g*) It has been claimed by Rippel and Poschenrieder (47) and by others that if considerable energy is required for nitrogen fixation the plant might, presumably, photosynthesize faster and replace the depleted supply. There is little to indicate that such is the case. To the contrary, legumes supplied with fixed nitrogen, as pointed out under (*d*), are more likely to grow at a slightly lower carbohydrate level than do the nodule-bearing legumes. If plants have the ability to speed up photosynthesis would they not do so in the plant having the lowest amount of carbohydrate, in the one receiving combined nitrogen, rather than in the one fixing nitrogen?

(*h*) Ammonium nitrate was used as the nitrogen source by Christiansen-Weniger and by Allam, and theoretically 1.3 gm. of sugar is required at 100 per cent efficiency to reduce 1 gm. of nitrate nitrogen to ammonia. According to Burk (9) the efficiency of nitrate reduction is probably not lower than about 32 per cent. About 4 gm. of sugar would then be required to reduce 1 gm. of nitrate nitrogen to ammonia, or 2 gm. to convert 1 gm. of ammonium nitrate nitrogen to ammonia. This affects the final dry weights of the plants to the extent of, at most, only about 4 per cent.

A number of other points of lesser importance might be mentioned, but certainly these are adequate to show that it is practically impossible to determine accurately the energy requirements in nitrogen fixation by the methods previously used. Of the foregoing eight points the first six and probably seven would ordinarily tend to make the final result show a considerable apparent energy requirement for fixation. It should be emphasized, however, that the results of Christiansen-Weniger show a very small maximum requirement, regardless of the points mentioned, and this is exceedingly strong evidence that the requirements for chemical fixation are negligible.

In the foregoing, calculations based on the findings of Newton (37) and of Reinau (45) indicated that the energy requirements of the nodule bacteria for both respiration and nitrogen fixation are probably not greater than about 3 to 6 per cent of the total carbohydrates photosynthesized. These data also indicate that the energy requirements for nitrogen fixation are small.

BACTERIAL-HOST RELATIONSHIP

Many workers in the past have considered that perhaps a type of physiological balance exists between legume nodule bacteria and their hosts under normal conditions. The Hiltner (19) and Suchting (51) immunity theories, for example, were advanced, but these no longer seem plausible. Wunschick (60) and others [*see* review by Allen and Baldwin (2)] have more recently suggested that there is an equilibrium between the vegetative energy of the higher plant and that of the bacteria.

The newer evidence, together with the old, tends to place much greater emphasis on carbohydrate nutrition in so far as effective bacterial strains are concerned. If the carbohydrate supply is adequate, nodules usually develop and nitrogen fixation roughly parallels the growth of the higher plant; if the supply then becomes deficient, the bacteria sometimes remain more or less dormant in the nodule, or in other cases may attack the tissues of the host to obtain food. As has been pointed out, many factors other than carbohydrate, such as calcium deficiency, high acidity, and insufficient soil moisture, may often markedly interfere with nodule formation and nitrogen fixation, but under reasonably normal soil conditions the carbohydrate supply seems to be the primary factor governing the intimate bacterial-host relations in legume symbiosis.

SUMMARY

This paper considers the rôle that carbohydrate plays in legume symbiosis from the time of bacterial entrance into the root until the nodule disintegrates, special emphasis being placed upon the response of the bacteria and host to varying supplies of carbohydrate. The more important facts gathered from many sources, which have a bearing on the subject, are brought together and correlated.

Nodule bacteria enter plant roots under almost any condition of carbohydrate concentration, but good nodule development is dependent upon a rather abundant carbohydrate supply in the roots.

The manner in which the carbohydrate is utilized, as the rate of photosynthesis varies, is considered in detail. If the carbohydrate is very limited, most of it is used for respiration, but as the supply increases growth becomes more abundant. At a carbohydrate level sufficiently high to permit considerable root growth, good nodule development normally takes place. If carbohydrates are supplied to the root, an abnormally large development of roots and nodules with respect to tops may occur.

The location of nodules is affected by the carbohydrate supply. Normally, nodules on annual legumes are located chiefly in the vicinity of the main roots and near the surface, assuming uniform inoculation and good soil conditions. On the other hand, they are usually widely scattered on plants grown either in water cultures, or in a nitrogen-free atmosphere, or in increased carbon dioxide, and when produced by ineffective strains. In all of these cases carbohydrate is abundant throughout the root system.

The physiological condition of the nodule, whether of the healthy, nitrogen fixing type, or whether at the stage where disintegration is starting, is also dependent in a large measure upon the carbohydrate supply. Other factors such as disease may, of course, produce nodule decay in the presence of abundant carbohydrate. The probable reasons why legume bacteria do not ordinarily attack nodule tissue well supplied with carbohydrate are considered.

The failure of nodules to develop abundantly in the presence of excess available nitrogen is due primarily to decreased carbohydrate supply in the roots. Under these conditions the carbohydrate-nitrogen ratio is narrow and an unusually large percentage of the carbohydrate synthesized is used for top growth, leaving less for the growth of roots and nodules. Good nodule development takes place only provided there is adequate carbohydrate for good root growth.

The quantity of nitrogen fixed by legumes growing under conditions favorable for fixation, usually, but not always, varies closely with the carbohydrate supply; the fixation roughly parallels the growth of the host tissue.

A consideration of the limited data, dealing with the energy requirements for the chemical process of nitrogen fixation as distinguished from the respiration and growth requirements of the bacteria and host, shows with a fair degree of certainty that they are negligible. The bulk of the carbohydrate appears to be consumed in respiration and growth, chiefly of the host.

These studies show very definitely that under reasonably normal conditions and with effective strains, the intimate relations existing between the nodule bacteria and their hosts in legume symbiosis are largely dependent upon the carbohydrate supply. If the supply is adequate, normal nodule activity usually follows; if the supply then becomes deficient, the bacteria sometimes merely remain more or less dormant in the nodule, or in other cases may attack the tissues of the host to obtain food. Under special conditions other factors, such as calcium deficiency, high acidity, and insufficient soil moisture, may, of course, outweigh carbohydrate supply in importance.

REFERENCES

(1) ALLAM, FLATHALLA 1931 Vom Energieverbrauch der Knöllchenbakterien bei der Bindung des Luftstickstoffs. *Ztschr. Pflanzenernähr, Düngung, u. Bodenk.* 20: 270–301.

(2) ALLEN, O. N., AND BALDWIN, I. L. 1931 The effectiveness of rhizobia as influenced by passage through the host plant. *Wis. Agr. Exp. Sta. Res. Bul.* 106: 1–56.

(3) ALLISON, FRANKLIN E., AND LUDWIG, CLINTON A. 1934 The cause of decreased nodule formation on legumes supplied with abundant combined nitrogen. *Soil Sci.* 37: 431–443.

(4) ALMON, LOIS 1933 Concerning the reproduction of bacteroids. *Centbl. Bakt.* (II) 87: 289–297.

(5) ARTHUR, JOHN M., GUTHRIE, JOHN D., AND NEWELL, JOHN M. 1930 Some effects of artificial climates on the growth and chemical composition of plants. *Jour. Bot.* 17: 416–482.

(6) BARTHEL, C. 1930 The growth of *Bact. radicicola* under reduced oxygen pressure. *Proc. Second Internatl. Cong. Soil Sci.* (III): 72–73.

(7) BRENCHLEY, W. E., AND THORNTON, H. G. 1925 The relation between the development, structure and functioning of the nodules on *Vicia faba*, as influenced by the presence or absence of boron in the nutrient medium. *Proc. Roy. Soc.* (B) 98: 373–399.

(8) BURK, DEAN 1930 The energy and chemical mechanism of nitrogen fixation by Azotobacter. *Proc. Second Internatl. Cong. Soil Sci.* (III): 67–71.

(9) BURK, DEAN 1931 The reversibility of coupled reactions in biological systems and the second law of thermodynamics. *Jour. Phys. Chem.* 35: 432–455.

(10) CHRISTIANSEN-WENIGER, F. 1923 Der Energiebedarf der Stickstoffbindung durch die Knöllchenbakterien im Vergleich zu anderen Stickstoffbindungs-möglichkeiten und erste Versuche zur Ermittlung desselben. *Centbl. Bakt.* (II) 58: 41–66.

(11) DUNHAM, D. H., AND BALDWIN, I. L. 1931 Double infection of leguminous plants with good and poor strains of rhizobia. *Soil Sci.* 32: 235–249.

(12) EATON, SCOTT V. 1931 Effects of variations in day-length and clipping of plants on nodule development and growth of soybean. *Bot. Gaz.* 91: 113–143.

(13) FRANK, B. 1890 Ueber die Pilzsymbiose der Leguminosen. *Landw. Jahrb.* 19: 523–640.

(14) FRED, E. B., BALDWIN, I. L., AND MCCOY, E. 1932 Root nodule bacteria and leguminous plants. *Univ. Wis. Studies Sci.* 5: 1–343.

(15) FRED, E. B., AND GRAUL, E. J. 1916 The effect of soluble nitrogenous salts on nodule formation. *Jour. Amer. Soc. Agron.* 8: 316–328.

(16) GEORGI, C. E., ORCUTT, F. S., AND WILSON, P. W. 1933 Further studies on the relation between the carbon assimilation and nitrogen fixation in leguminous plants. *Soil Sci.* 36: 375–382.

(17) GIÖBEL, GUNNAR 1926 The relation of soil nitrogen to nodule development and fixation of nitrogen by certain legumes. N. J. Agr. Exp. Sta. Bul. 436.

(18) GOLDING, JOHN 1899 Sugar as an agent in nitrogen fixation and an aid to the growth of plants. *Jour. Soc. Chem. Indus.* 18: 564–566.

(19) HILTNER, L. 1900 Ueber die Ursachen, welche die Grösse, Zahl, Stellung und Wirkung der Wurzelknöllchen der Leguminosen bedingen. *Arb. K. Gsndhtsamt. Biol. Abt.* 1: 177–222.

(20) HILTNER, L., AND STÖRMER, K. 1903 Neue Untersuchungen über die Wurzelknöllchen der Leguminosen und deren erreger. *Arb. K. Gesndhtsamt. Biol. Abt.* 3: 151–307.

(21) HOPKINS, E. W., AND FRED, E. B. 1933 Influence of various nitrogenous compounds and mannitol on nodule formation by clover. *Plant Physiol.* 8: 141–155.

(22) JONES, F. R., AND TISDALE, W. B. 1921 Effect of soil temperature upon the development of nodules on the roots of certain legumes. *Jour. Agr. Res.* 22: 17–31.
(23) KNUDSON, L. 1916 Influence of certain carbohydrates on green plants. *Cornell Agr. Exp. Sta. Mem.* 9: 1–75.
(24) KOSSOWITSCH, P. 1892 Durch welche Organe nehmen die Leguminosen den freien Stickstoff auf? *Bot. Ztg.* 50: 697–702, 713–723, 729–738, 745–756, 771–774.
(25) LAURENT, E. 1891 Recherches sur les nodosités radicales des Légumineuses. *Ann. Inst. Pasteur* 5: 105–139.
(26) LEONARD, L. T. 1926 A preliminary note on the relation of photosynthetic carbohydrate to nodule formation on soybeans. *Jour. Amer. Soc. Agron.* 18: 1012–1016.
(27) LEWIS, KEITH H., AND MCCOY, ELIZABETH 1933 Root nodule formation on the garden bean, studied by a technique of tissue culture. *Bot. Gaz.* 95: 316–329.
(28) LÖHNIS, MARIE P. 1930 Investigations upon the ineffectiveness of root-nodules on Leguminosae. *Centbl. Bakt.* 80: 342–368.
(29) LUNDEGÅRDH, HENRIK. 1924 Der Kreislauf der Kohlensäure in der Natur. Ein Beitrag zur Pflanzenökologie und zur landwirtschaftlichen Düngungslehre. Jena.
(30) MCCOY, ELIZABETH F. 1929 A cytological and histological study of the root nodules of the bean, *Phaseolus vulgaris* L. *Centbl. Bakt.* (II) 79: 394–412.
(31) MCCOY, ELIZABETH F. 1932 Infection by *Bact. radicicola* in relation to the microchemistry of the hosts' cell walls. *Proc. Roy. Soc. Ser.* B, 110: 514–533.
(32) MASON, T. G., AND MASKELL, E. J. 1928 Studies on the transport of carbohydrates in the cotton plant. *Ann. Bot.* 42: 189–253, 571–636.
(33) MILLER, EDWIN C. 1931 Plant Physiology. McGraw-Hill Book Co., New York.
(34) MILOVIDOV, P. 1925 Bactéries des tubercules radicaux de certaines légumineuses. *Pub. Facult. Sci. Univ. Charles á Prague* 49: 1–40.
(35) MÜLLER, A., AND STAPP, C. 1925 Beiträge zur Biologie Leguminosenknöllchenbakterien mit besonderer Berücksichtigung ihrer Artverschiedenheit. *Arb. Biol. Reichsanst. Land u. Forstw.* 14: 455–554.
(36) MÜLLER, D. 1932 Analyse der verminderten Stoffproduktion bei Stickstoffmangel. *Planta* (*Abt. E, Ztschr. Wiss. Biol.*) 16: 1–9.
(37) NEWTON, J. D. 1923 A comparison of the absorption of inorganic elements, and of the buffer systems of legumes and non-legumes and its bearing upon existing theories. *Soil Sci.* 15: 181–204.
(38) NOBBE, F., AND HILTNER, L. 1899 Über die Wirkung der Leguminosenknöllchen in der Wasserkultur. *Landw. Vers. Sta.* 52: 455–65.
(39) NOBBE, F., SCHMID, E., HILTNER, L., AND HOTTER, E. 1892 Über die Verbreitungsfähigkeit der Leguminosen-Bakterien im Boden. *Landw. Vers. Sta.* 41: 137–138.
(40) OTIS, D. H. 1898 Root tubercles and their production by inoculation. *Industrialist* 24: 363–378.
(41) PEIRCE, GEORGE JAMES 1902 The root-tubercles of bur clover (*Medicago denticulata*, willd.) and of some other leguminous plants. *Proc. Calif. Acad. Sci., 3d Ser.* (*Botany*) 2: 295–328.
(42) PRUCHA, MARTIN J. 1915 Physiological studies of *Bacillus radicicola* of Canada field pea. *Cornell Agr. Exp. Sta. Mem.* 5: 1–83.
(43) REINAU, E. H. 1924 Woher stammen die deutschen Ernten? Ein Beitrag zur Frage der Bodenkohlensäure. *Technik Landw.* 10: 182–196.
(44) REINAU, E. 1926 Der kleine Kreislauf der Kohlensäure beim Anbau von Leguminosen. *Fortschr. Landw.* 1: 787–789.
(45) REINAU, E. 1927 Praktische Kohlensäure-düngung in Gärtnerei und Landwirtschaft. Berlin.
(46) RIEDELS, F. 1922 Kohlensäuredüngung. *Technik. Landw.* 3: 87–89.
(47) RIPPEL, A., AND POSCHENRIEDER, H. 1928 Prinzipielle Bemerkungen zur Stickstoffbindung durch Mikroörganismen. *Jour. Landw.* 76: 101–112.

(48) RÜFFER, ERNST 1932 Forschungen zum Kohlenhydratumsatz bei knöllschentragenden und knollchenfreien Sojabohnen. *Ztschr. Pflanzenernähr, Düngung, u. Bodenk.* 20: 270–301.

(49) SCHAEDE, REINHOLD 1932 Das Schicksal der Bakterien in den Knöllchen von *Lupinus albus* nebst cytologischen Untersuchungen. *Centbl. Bakt.* (II) 85: 416–425.

(50) SPOEHR, H. A. 1926 Photosynthesis. Amer. Chem. Soc. Monog. Ser. The Chemical Catalog Co., New York.

(51) SÜCHTING, H. 1904 Kritische Studien über die Knöllchenbakterien. *Centbl. Bakt.* (II) 11: 377–388, 417–441, 496–520.

(52) THORNTON, H. G. 1930 The early development of the root nodule of lucerne (*Medicago sativa*, L.). *Ann. Bot.* 44: 385–392.

(53) THORNTON, H. G. 1930 The influence of the host plant in inducing parasitism in lucerne and clover nodules. *Proc. Roy. Soc.* (B) 106: 110–122.

(54) TRANSEAU, E. N. 1926 The accumulation of energy by plants. *Ohio Jour. Sci.* 26: 1–10.

(55) WEBER, ERWIN 1930 Salpeterdüngung als Beinträchtigung der Stickstoffsammlung durch Leguminosen. *Centbl. Bakt.* (II) 82: 353–379.

(56) WHITING, A. L. 1915 A biochemical study of nitrogen in certain legumes. *Ill. Agr. Exp. Sta. Bul.* 179: 471–542.

(57) WILSON, J. K. 1917 Physiological studies of *Bacillus radicicola* of soybean (*Soja max* Piper) and of factors influencing nodule production. *Cornell Agr. Exp. Sta. Bul.* 386: 369–413.

(58) WILSON, J. K. 1931 Nodule production on etiolated vetch seedlings. *Phytopath.* 21: 1083–1085.

(59) WILSON, P. W., FRED, E. B., AND SALMON, M. R. 1933 Relation between carbon dioxide and elemental nitrogen assimilation in leguminous plants. *Soil Sci.* 35: 145–165.

(60) WUNSCHICK, H. 1925 Erhöhung der Wirksamkeit der Knöllchenerreger unserer Schmetterlingsblütler durch Passieren der Wirtspflanze. *Centbl. Bakt.* (II) 64: 395–445.

A RESPONSE OF CHLOROTIC CORN PLANTS TO THE APPLICATION OF ZINC SULFATE TO THE SOIL[1]

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The physiological disease of corn locally called "white bud," a form of chlorosis, is widely distributed in central Florida. It is found chiefly in the fields which have been under cultivation for a number of years, but in some instances occurs on the poorer types of recently cleared land. It is widely distributed on the lighter sandy soils but also occurs on the heavier soils when they are subjected to excessive cultivation and continuous culture. The soils on which this injury has been observed to develop on corn include the Norfolk, Gainesville, Hernando, and Blanton series. In some fields the injury has been so acute that corn has failed completely unless large quantities of animal manures were used or the land was allowed to lie idle and grow up in weeds for a number of years. An extreme case of this trouble exists on the Florida Experiment Station farm at Gainesville. Here, on Norfolk medium fine sand and on Hernando medium fine sand, "white bud" has been exceptionally serious for the past several years. The land on this farm has been under continuous culture for years. Neither cover crops nor liberal applications of mixed commercial fertilizers have materially improved the chlorotic condition of the corn.

DESCRIPTION OF "WHITE BUD" OR CHLOROSIS OF CORN

Within a week after the emergence of the corn seedlings growing in affected soil, symptoms of chlorosis begin to show. The full development of chlorophyll in the older leaves scarcely takes place before light yellow streaks appear between the veins. Small white spots of inactive or dead tissue develop rapidly in the leaves, while small white areas which never develop chlorophyll are sometimes prevalent. The unfolding leaves in the buds of corn seedlings are often white to a very light yellow in color, giving rise to the use of the term "white bud" by farmers. These symptoms are shown in plate 1, figure 1.

On exceptionally bad areas, the corn seedlings seldom recover from the chlorosis. The older leaves die, and yellow to white leaves continue to unroll. Before the older leaves die they develop a number of dead areas ranging in color from light slate to dark brown. These areas often grow larger and merge until the whole leaf dies. Plate 1, figure 2 shows such a corn plant.

[1] Publication authorized by the director of the Florida Agricultural Experiment Station. Contribution from the department of chemistry and soils and the department of agronomy.

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Other advanced stages of chlorosis or "white bud" are illustrated in plate 1, figures 3 and 4. Figure 3 (pl. 1) shows an entire corn plant in which the lower older leaves are practically dead, and in some places the leaf tissue has actually fallen away or contracted so as to give the leaves a distorted appearance. The upper leaves show typical yellow striping between the veins. The internodes of the plant are definitely shortened, and the growth is stunted. It is apparent that this diseased corn plant, which is already tasseling, will probably not produce a healthy ear of corn. Figure 4 (pl. 1) illustrates some of the advanced stages of injury to the corn leaves as compared with the appearance of a healthy leaf. These more advanced symptoms of the chlorosis are usually found in the lower leaves of the affected plants. These leaves, in most cases, die long before the maturity of the plant.

Examination of the root systems of chlorotic and apparently healthy plants at different stages of growth have failed to reveal any outstanding differences. Both "white bud" and healthy plants evidently develop healthy root systems during the early stages of growth.

Field tests[3] with different varieties and selections of corn have shown that the varieties and selections developed on the poorer sandy soils are more resistant to "white bud" than those developed on the more fertile soils. However, even the more resistant varieties become chlorotic on the worst affected areas.

The date of planting does not appear to have any definite effect on the ultimate development of chlorosis in the corn plant. Corn planted in February and March grows slower than that planted in April, May, and June, and for this reason the symptoms in the earlier plantings are more readily observed and are apparently more pronounced.

Although an entire field of corn may show symptoms of the chlorosis in varying degrees, there are usually areas in which the plants are more severely affected than in others. These areas are usually irregularly distributed over the field and are irregular in outline. To date no correlation has been observed between the occurrence of these localized severely affected areas and any soil characteristic.

Various types of pot cultures of corn in the surface soils and subsoils from affected areas were made in the greenhouse and in the open. City water was used on these plants. In no instance did the "white bud" condition develop in the corn plants of these cultures. Later, a quantity of the Gainesville city water was evaporated and the residue tested spectroscopically for the presence of some of the less abundant elements. Zinc was found in relatively large quantities in the residue. Traces of cadmium and copper were also detected. In view of the response of the "white bud" corn in the field to applications of zinc sulfate, as described in the following, it appears likely that the quantities

[3] The authors are indebted to Mr. F. H. Hull, associate agronomist of the Florida Agricultural Experiment Station, for making these observations. Mr. Hull has accumulated data on corn varieties and dates of planting on the Station Farm for a number of years.

of zinc found in the city water were sufficient to prevent the development of "white bud." The field experiments were started in the spring of 1933.

METHOD OF FIELD EXPERIMENTATION

Tests were made on the Station Farm in a field that had produced chlorotic corn plants very uniformly over its entire area for a number of years. The soil is classified as a Norfolk medium fine sand with some small areas of Hernando medium fine sand. Within this affected area 3-foot by 6-foot plots were laid out in tiers with a 4-foot border around each plot. Each plot was enclosed to a depth of 8 inches with 1-inch by 10-inch cypress boards nailed together to form a frame. The frames were sunk into the ground by digging a trench around the block of soil in which the treatments were to be made and slipping the frame down around the block. This method proved quite satisfactory for the isolation of the plots but necessitated hand cultivation. The pH value of the surface soil and subsoil of the plots ranged from 5.58 to 6.35.

Four tiers of 40 plots each were established in this manner. All special treatments were made in quadruplicate, being replicated in each tier of plots. In each tier, 10 plots were maintained without special treatment making a total of 40 check plots. The plots were located so as to cover the variations in the soil as far as possible.

The general plan of the experiment included two sets of treatments involving (*a*) a mixed inorganic fertilizer made of nitrate of soda, superphosphate, and muriate of potash with and without mineral supplements and (*b*) a variety of organic materials in some instances in combination with mineral supplements. The different treatments and their rates per acre are given in table 1.

EXPERIMENTAL RESULTS

On March 22, 1933, the fertilizer treatments, organic materials, and supplements were applied in a row lengthwise of the small plots except where otherwise indicated. They were thoroughly incorporated with the soil in the row with a hoe. The 8–8–4 mixed inorganic fertilizer was made from nitrate of soda, superphosphate, and muriate of potash. It was applied uniformly to the plots receiving inorganic combinations at the rate of 400 pounds per acre. All materials were of the commercial grade used in fertilizers except the zinc sulfate, which was the chemically pure "analyzed salt" used in chemical laboratories. Only one-quarter of the nitrogen of the mixed inorganic fertilizer was applied at the time of the planting. The remainder was used as a side-dressing. On the same date nine Whatley Prolific corn seeds were planted in the row in each plot. Germination counts were made on April 6. Because of some injury to germination by certain treatments, the seedlings were removed from all plots on April 7, and the plots were replanted to the same variety of corn. Germination of this planting was satisfactory, and the young seedlings made a good start. Table 1 gives the treatments used and the germination percentages obtained from counts on the two plantings. In the first planting the

TABLE 1

Treatments applied to soil in studies of chlorosis or "white bud" of corn and their effect upon the germination of corn

TREATMENT NUMBER	MATERIALS APPLIED TO SOIL PER ACRE	PER CENT GERMITION	
		First test (a)	Second test (b)
1	Nothing* No special treatment	76.6	88.6
	Mixed inorganic fertilizer and supplements†		
2	8–8–4, Nitrate of soda, superphosphate, muriate of potash	61.1	94.4
3	8–8–0 + 200 pounds muriate of potash	44.4	94.4
4	8–8–0 + 375 pounds sulfate of potash-magnesia	36.1	88.9
5	8–8–4 + 400 pounds epsom salts	47.2	83.3
6	8–8–4 + 50 pounds manganous sulfate	66.6	94.4
7	8–8–4 + 200 pounds manganous sulfate	72.2	88.9
8	8–8–4 + 1,000 pounds dolomitic limestone	63.9	94.4
9	8–8–0 + 200 pounds muriate of potash and 200 pounds manganous sulfate	38.9	97.2
10	8–8–4 + 20 pounds zinc sulfate ($ZnSO_4 \cdot 7H_2O$)	77.8	80.6
11	8–8–4 + 500 pounds flowers of sulfur	83.3	91.7
12	8–8–4 + 500 pounds flowers of sulfur and 50 pounds manganous sulfate	63.9	94.4
13	8–8–4 + 1,000 pounds basic slag	83.3	86.1
14	8–8–0 + 375 pounds sulfate of potash-magnesia and 200 pounds manganous sulfate	47.2	86.1
15	0–8–4 + 50 pounds manganous sulfate and 178 pounds calcium nitrate	86.1	88.9
	Organic materials and supplements†		
16	2½ tons air-dried chlorotic *Crotalaria spectabilis* (broadcast)	72.2	94.4
17	2½ tons air-dried healthy *Crotalaria spectabilis* (broadcast)	72.2	94.4
18	2½ tons natural leaf mold from mixture of hardwoods and pine (broadcast)	77.8	83.3
19	2½ tons natural leaf mold (broadcast) and 50 pounds manganous sulfate (in row)	88.9	88.9
20	4 tons stable manure (horse)	63.9	88.9
21	2 tons chicken manure (without litter)	77.8	94.4
22	400 pounds Peruvian guano	69.4	88.9
23	5 tons acid peat from localized peat deposit near LaCrosse, Fla.	80.6	83.3
24	5 tons acid peat and 50 pounds of manganous sulfate	77.8	80.6
25	5 tons alkaline peat from Everglades Experiment Station, Belle Glade, Fla.	83.3	83.3
26	5 tons alkaline peat and 50 pounds manganous sulfate	72.2	91.7
27	5 tons alkaline peat and 20 pounds zinc sulfate ($ZnSO_4 \cdot 7H_2O$)	83.3	86.1
28	5 tons alkaline peat and 1,000 pounds basic slag	86.1	86.1

(a) Whatley's Prolific corn. Planted March 22, 1933. Germination count made April 6, 1933.

(b) Whatley's Prolific corn. Planted April 7, 1933. Germination count made April 19, 1933.

* Average of 40 plots. † Average of quadruplicated plots for each treatment.

germination of the corn seed was seriously injured by the larger applications of soluble magnesium and potassium salts and to a lesser extent by some of the other treatments. No injury to germination was observed in the second planting made approximately 2 weeks after the application of the materials.

TABLE 2

Percentage of corn plants showing "white bud" condition on plots receiving different treatments on dates indicated

TREATMENT NUMBER*	PER CENT CHLOROTIC PLANTS					
	April 19	April 24	April 28	May 2	May 6	May 8
1	85.8	97.8	94.9	94.2	100.0	100.0
2	67.6	100.0	58.9	41.2	94.0	100.0
3	86.7	100.0	55.9	61.9	100.0	100.0
4	87.4	100.0	62.5	68.8	96.9	100.0
5	86.7	96.5	55.2	41.4	96.6	100.0
6	91.2	100.0	47.0	23.5	100.0	97.0
7	96.8	100.0	53.1	43.8	84.4	100.0
8	85.2	96.9	63.3	50.0	100.0	100.0
9	91.4	100.0	63.7	45.4	90.9	100.0
10	86.2	100.0	81.5	33.3	22.2	3.7
11	69.7	84.9	48.5	27.3	81.3	96.8
12	85.2	94.4	54.3	54.3	100.0	100.0
13	90.4	100.0	29.0	41.9	100.0	100.0
14	93.6	100.0	28.1	12.5	37.5	68.7
15	90.7	96.9	40.7	43.7	96.8	100.0
16	85.2	100.0	66.7	90.9	100.0	100.0
17	67.6	100.0	100.0	85.3	100.0	100.0
18	83.3	100.0	74.2	58.0	76.7	56.7
19	71.9	96.9	21.9	41.9	54.5	64.5
20	71.9	9.4	0.0	21.9	25.0	0.0
21	64.7	64.7	35.3	67.6	66.7	84.8
22	90.7	100.0	96.8	100.0	100.0	100.0
23	86.7	100.0	86.2	86.2	100.0	100.0
24	82.7	96.5	53.6	35.7	96.3	92.9
25	96.6	100.0	43.3	40.0	87.0	80.0
26	87.9	96.9	35.5	35.5	90.3	83.9
27	93.6	100.0	40.2	12.9	51.7	3.3
28	77.4	90.0	6.7	46.7	100.0	100.0

* For materials applied to soil in each treatment, see table 1.

A number of observations were made on the occurrence of the chlorosis or "white bud" among the corn plants during the early stages of growth. The total number of plants and the number of chlorotic plants were counted. The results of these counts were calculated to percentages and are given in table 2. A large percentage of the very young corn seedlings were chlorotic, as seen from the count of April 19. Only the plants in the stable manure plots showed defi-

nite improvement by April 24. On April 28, the plants of the plots receiving an application of stable manure showed no chlorotic plants. A number of other treatments brought about a temporary improvement in plant condition during this period, but only the zinc sulfate supplement to alkaline peat and

TABLE 3

Average height of corn plants in plots receiving different treatments on dates indicated*

TREATMENT NUMBER†	HEIGHT IN FEET	
	June 2	June 29
1	0.8	3.1
2	0.7	2.8
3	0.9	3.4
4	1.2	4.3
5	1.1	3.9
6	1.0	3.7
7	1.0	3.5
8	0.8	3.0
9	1.1	4.2
10	1.7	5.9
11	0.8	1.9
12	0.9	3.3
13	0.6	2.6
14	1.7	5.6
15	1.1	4.0
16	0.9	3.4
17	0.9	3.9
18	1.4	5.7
19	1.7	5.9
20	2.8	7.0
21	2.3	6.6
22	0.9	3.6
23	0.9	3.1
24	1.1	4.0
25	1.4	4.7
26	1.5	4.9
27	1.9	6.4
28	1.1	4.2

* Whatley's Prolific corn, planted, April 7, 1933.

† For materials applied to soil in each treatment, see table 1.

the application of stable manure caused a plant improvement which was not transitory.

On May 2, the general condition of most of the plots receiving special treatment was relatively good. The plants of the plot receiving the zinc sulfate supplement to the mixed inorganic fertilizer application showed definite improvement for the first time on this date. On May 6, the plants showing the

least amount of the chlorosis were those in the plots receiving applications of the mixed inorganic fertilizer with 20 pounds per acre of zinc sulfate, the mixed inorganic fertilizer using sulfate of potash-magnesia and 200 pounds per acre of manganous sulfate, the stable manure plots and the alkaline peat plots plus 20 pounds of zinc sulfate per acre. The plants in plots treated with chicken manure and those treated with leaf mold showed a definite decrease in the amount of "white bud" during this period. On May 8, the plants treated with zinc sulfate and those treated with stable manure showed little or no chlorosis. The plants of the plots receiving an application of the mixed inorganic fertilizer with the potash from sulfate of potash-magnesia and 200 pounds per acre of manganous sulfate and those of the plots receiving applications of leaf mold showed definite improvement. The plants growing in plots treated with chicken manure also showed great improvement. They grew vigorously but during this period showed some evidence of "white bud."

After the count of the total number of the chlorotic plants on May 8, the stand was thinned to three plants per plot. A side-dressing of nitrate of soda was made to the plants receiving the mixed inorganic fertilizer on May 22. Height measurements of the individual corn plants in the plots were made on June 2 and June 29. The average heights of the corn plants in the differently fertilized and treated plots are given in table 3 for these dates.

From the height data of table 3, it may be seen that the application of 20 pounds per acre of zinc sulfate ($ZnSO_4 \cdot 7H_2O$) with 400 pounds per acre of an 8–8–4 inorganic fertilizer made from nitrate of soda, superphosphate, and muriate of potash greatly stimulated the growth of the corn plants. The only other inorganic fertilizer combination which significantly stimulated the corn growth was the application of 375 pounds per acre of sulfate of potash-magnesia and 200 pounds per acre of manganous sulfate with the mixed inorganic fertilizer. Leaf mold, stable manure, and chicken manure produced vigorous plants as did 20 pounds of zinc sulfate in combination with alkaline peat at the rate of 5 tons per acre. Plate 2 illustrates the effect of the zinc sulfate in combination with a mixed inorganic fertilizer and with alkaline peat.

The corn plants were allowed to mature completely and the ears were husked from the stalks of each plot on September 11. The corn stalks were not harvested. The corn on the cob was thoroughly dried for 5 days at 100°C. The weights of the dried grain and cobs were obtained. The total number of stalks, the total number of ears, and the total dry weight of the corn produced on the quadruplicated plots of like treatment are given in table 4.

Among the completely inorganic combinations, the zinc sulfate supplement and the potash-magnesia-manganous sulfate combination with 400 pounds per acre of the inorganic 8–8–4 mixed fertilizer gave the largest and most significant grain yield. The zinc sulfate supplement gave much higher grain yields than the potash-magnesium sulfates with manganous sulfate. Stable manure, at the rate of 4 tons per acre applied in the row, increased the corn yield more than the other organic additions. Among the organic materials, alkaline

peat at the rate of 5 tons per acre with the zinc sulfate supplement was the most effective treatment in increasing the corn yield. Two and one-half tons per acre of leaf mold with 50 pounds per acre of manganous sulfate, 2 tons per acre of chicken manure, 2½ tons per acre of leaf mold, 5 tons of alkaline peat with

TABLE 4

The total yield of corn grown on quadruplicated plots

(Dried at 100°C.)

TREATMENT NUMBER*	NUMBER		TOTAL WEIGHT		
	Stalks	Ears	Grain	Cobs	Grain & Cob
			gm.	*gm.*	*gm.*
1	11	6	204.6	57.9	262.5
2	8	2	51.0	17.0	68.0
3	11	3	59.0	28.0	87.0
4	11	12	462.0	128.0	590.0
5	11	10	345.0	93.0	438.0
6	12	9	409.0	108.0	517.0
7	12	8	167.0	51.0	218.0
8	10	3	105.0	28.0	133.0
9	11	9	298.0	78.0	376.0
10	12	18	983.0	252.0	1235.0
11	8	4	195.0	61.0	256.0
12	11	7	137.0	44.0	181.0
13	7	0	—	—	—
14	12	15	715.0	191.0	906.0
15	12	9	479.0	109.0	588.0
16	10	6	320.0	86.0	406.0
17	8	6	253.0	75.0	328.0
18	12	16	926.0	228.0	1154.0
19	12	18	1092.0	261.0	1353.0
20	12	22	1328.0	360.0	1688.0
21	12	22	955.0	397.0	1352.0
22	9	8	250.0	73.0	323.0
23	11	3	78.0	27.0	105.0
24	12	10	396.0	129.0	525.0
25	12	14	669.0	160.0	829.0
26	12	17	797.0	188.0	985.0
27	12	20	1261.0	277.0	1538.0
28	11	10	362.0	114.0	476.0

* For materials applied to soil in each treatment, see table 1.

50 pounds per acre of manganous sulfate were the other organic materials, (named in decreasing order), which materially and definitely increased the corn yields. The application of 1,000 pounds of basic slag per acre in the planting furrow brought about an exaggeration of the "white bud" condition when used either with a mixed inorganic fertilizer or with alkaline peat.

REVIEW OF LITERATURE AND DISCUSSION

The importance of the less abundant elements in life processes has been recognized for a long time. With the full appreciation of the importance of these elements in biologic processes, the study of their distribution and function has been expanded until today an extensive literature has developed. For this reason, only a few of the publications treating of the zinc response of annual plants will be reviewed. Studies covering more specifically the zinc response of the corn and similar plants will be emphasized.

Brenchley (3) in 1927 gave an excellent review of the early reports on the effect of zinc on plant growth. Among these reports, the water culture studies of Mazé (8) and the field experiments of Javillier (5) on the effect of zinc compounds on the corn plant are of particular value for the present study.

Mazé (8) started the study of the effect of small amounts of several of the less abundant elements on the development of the corn plant in solution cultures in 1910, and continued them through several succeeding years. He used especially treated containers and purified salts. His early studies with zinc were evidently not very satisfactory but he reported that he obtained characteristic zinc-deficient plants in 1912. He described the leaves of the corn plants growing without a source of zinc as being darkened in color and having a metallic sheen. Nocturnal sweating became abundant, leaving deposits on the leaves and causing an incrustation. Soluble salts were found in the deposits, and the zinc-deficient plants were evidently not able to regulate the absorption of nutrients. The plants died from 3 to 5 days after the appearance of these symptoms. The corn plants grown in solutions without a source of zinc lived from 4 to 6 weeks after being placed in the solutions.

Among his numerous contributions to the zinc response in plants, Javillier (5), in 1912, reported field experiments in which crystallized zinc sulfate was applied simultaneously with appropriate fertilizers to wheat, corn, oats, lupine, and peas. One to ten kilograms (2.2 to 22 pounds of zinc sulfate per hectare (2½ acres) were used. Uniformly favorable results from the application of zinc sulfate to corn were obtained in the tests. The zinc sulfate increased the dry weight of the immature corn plants 18 to 25 per cent in some instances, while a 5 per cent increase was the smallest obtained. Results with other plants were irregular, though in some instances wheat and lupine responded favorably to applications of zinc sulfate.

From studies carried out in solution cultures under very carefully controlled conditions, Sommer and Lipman (10) added the sunflower and barley to the plants for whose healthy growth zinc is apparently essential. Sommer (9) added buckwheat, Winsor beans, and red kidney beans to the list of plants for whose healthy growth zinc is evidently essential in solution cultures. Miss Sommer points out that up to the present there are two plants of the Gramineae, two plants of the Leguminosae, and one each of the Compositae and Polygenaceae families which give evidence of the essential nature of zinc for their

healthy growth in solution cultures. McHargue and Shedd (7) studied the cumulative effect of compounds of manganese, copper, zinc, boron, and arsenic on the growth of the oat plant in sand cultures. They concluded that there was an increase in the yield of grain and straw when compounds of manganese, copper, and zinc were used as compared to the use of manganese compounds alone.

Allison, Bryan, and Hunter (2) reported transitory favorable response of a number of plants growing in the raw saw-grass peat of the Florida Everglades to the application of zinc sulfate. They suggested the possible use of zinc compounds in connection with compounds of the other less abundant elements. Acting upon this suggestion, Allison (1) reported a favorable response of the peanut plant in its first growth stages to applications of zinc sulfate to the raw Everglades saw-grass peat. However, the initial response in plant growth from zinc sulfate did not continue and the plants soon broke down. At the same time, it was possible to bring the peanut plants to an earlier maturity with the addition of zinc sulfate and copper sulfate together than with copper sulfate alone. The zinc sulfate and copper sulfate were used at the rate of 16 pounds and 50 pounds per acre, respectively, in the planting row.

The toxicity of zinc compounds in relatively low concentrations to higher plants has been abundantly established in simple solutions, nutrient solutions, pot cultures (especially with galvanized pots), and in soils contaminated with, or naturally containing, zinc compounds.

Some of the terms used to designate the favorable action of zinc compounds on plant growth are: the "catalytic" or "complementary" action, used by Javillier; the "essential" nature of zinc, implied by Mazé and Miss Sommer; the "stimulation of plant growth," preferred by Miss Brenchley; and the action of zinc in "precipitating soil toxins," suggested by Chandler, Hoagland, and Hibbard (4) in their study of the little leaf or rosette of deciduous and citrus trees in California. It must be admitted that even the very careful work of Mazé and Miss Sommer does not absolutely establish the essential nature of zinc for the healthy growth of all possible genetic variations of plants under all possible environmental conditions. Apparently, the nature of the favorable action of zinc on plant growth will remain unknown until more information has been accumulated.

Although the absolutely essential nature of zinc for the healthy growth of higher plants under all conditions may still be questioned, it must be admitted that many of the symptoms recorded by Mazé for his zinc-deficient corn plants have been further observed for the "white bud" corn plants under field conditions. Miss Sommer (9) reports that wheat and barley, members of the same plant family as corn, made a healthy growth in zinc-deficient nutrient solutions for about 2 weeks but subsequently stopped growing and gradually dried up. Field observations show that the response of corn seedlings suffering from "white bud" was delayed several weeks after the application of the zinc sulfate and the germination of the corn seeds.

It is apparent that the soil factors bringing about a condition of the corn plant called "white bud," a form of chlorosis, are not as yet known. However, the response of the young chlorotic corn plants to applications of zinc sulfate furnishes ample evidence that the presence of an available source of zinc or possibly closely associated elements will overcome the condition and lead to a healthy growth of the corn plant.

In the present studies[4] a qualitative spectroscopic test revealed the presence of minute quantities of magnesium, cadmium, lead, and copper in the "chemically pure" zinc sulfate. The manganous sulfate which gave a response when used in combination with a 375-pound per acre application of sulfate of potash-magnesia had detectable quantities of zinc when tested spectroscopically. On the other hand the double sulfate of potash-magnesia did not show the zinc line under the experimental conditions.

The acid peat, alkaline peat, stable manure, chicken manure, leaf mold, normal *Crotalaria spectabilis* and chlorotic *Crotalaria spectabilis* were ashed below 450°C., and the ashes were tested spectroscopically for the presence of zinc. All of these organic substances had detectable quantities of zinc in their ashes.

In the field tests with corn, stable manure, chicken manure, leaf mold, and alkaline peat brought about a definite favorable response of the chlorotic corn plants and an increase in yield. The acid peat and *Crotalaria spectabilis* were not effective. These differences in response suggest that there might be a difference in the availability of the zinc contained in the various organic materials. It is interesting to note in connection with the favorable response of chlorotic corn plants to applications of leaf mold that McHargue and Roy (6) report the presence of zinc in the leaves of 23 specimens of deciduous trees.

After the favorable response of the chlorotic corn to the applications of zinc sulfate to the soil was observed, leaves of affected corn plants were washed with a dilute zinc sulfate solution and injections into the stalks of badly affected plants were made. Neither of these treatments brought about a recovery of the affected plants.

SUMMARY

A chlorosis of the corn plant, locally called "white bud" is described.

Corn plants affected with "white bud" responded to a 20-pound per acre application of "chemically pure" zinc sulfate ($ZnSO_4 \cdot 7H_2O$) with a mixed inorganic fertilizer and with alkaline peat in the soil. The chlorotic plants regained their green color, made a healthy growth, and produced grain following the application of zinc sulfate.

Stable manure and leaf mold produced healthy corn plants in an affected

[4] All spectroscopic tests were made by Mr. L. H. Rogers, who worked under the direction of Prof. R. C. Williamson of the physics department, University of Florida, and of Dr. L. W. Gaddum of the Florida Agricultural Experiment Station. The authors wish to thank these persons for their hearty coöperation.

soil. Chicken manure and alkaline peat brought about a definite improvement in affected plants but did not cause a complete removal of "white bud" symptoms. Qualitative spectroscopic tests on the ashes of these organic materials showed the presence of zinc.

REFERENCES

(1) ALLISON, R. V. 1928 Everglades Experiment Station. *Ann. Rpt. Fla. Agr. Exp. Sta.* 1928: 111R–117R.

(2) ALLISON, R. V., BRYAN, O. C., AND HUNTER, J. H. 1927 The stimulation of plant response on the raw peat soils of the Florida Everglades through the use of copper sulphate and other chemicals. Univ. Fla. Agr. Exp. Sta. Bul. 190.

(3) BRENCHLEY, W. E. 1927 Inorganic Plant Poisons and Stimulants, ed. 2. Cambridge University Press. Fetter Lane, London. The Macmillan Co., New York.

(4) CHANDLER, W. H., HOAGLAND, D. R., AND HIBBARD, P. L. 1932 Little leaf or rosette in fruit trees, II. Effect of zinc and other treatments. *Proc. Amer. Soc. Hort. Sci.* 29: 255–263.

(5) JAVILLIER, M. 1912 Sur l'emploi du zinc comme engrais catalytique. *Internatl. Cong. Appl. Chem. Orig. Commun.* 15: 145–146.

(6) MCHARGUE, J. S., AND ROY, W. R. 1932 Mineral and nitrogen content of the leaves of some forest trees at different times in the growing season. *Bot. Gaz.* 94: 381–393.

(7) MCHARGUE, J. S., AND SHEDD, O. M. 1930 The effect of manganese, copper, zinc, boron and arsenic on the growth of oats. *Jour. Amer. Soc. Agron.* 22: 739–746.

(8) MAZÉ, P. 1914 Influences respectives des elements de la solution minerale sur le development du mais. Ann. Inst. Pasteur. 28: 21–68.

(9) SOMMER, A. L. 1928 Further evidence of the essential nature of zinc for the growth of higher green plants. *Plant Physiol.* 3: 217–221.

(10) SOMMER, A. L., AND LIPMAN, C. B. 1926 Evidence of the indispensable nature of boron and zinc for higher plants. *Plant Physiol.* 1: 231–249.

PLATE 1

CHLOROSIS OF CORN

FIG. 1. Early symptoms of "white bud" in seedling of Whatley's Prolific Corn. Planted April 7, 1933, photographed April 24, 1933.

FIG. 2. Advanced stage of "white bud" in young Whatley's Prolific corn plant. Plants seldom recover from this stage. Planted, March 6, 1933, photographed April 24, 1933.

FIG. 3. Chlorotic Long Island Beauty corn plant showing striping of leaves. Planted, February 4, 1933, photographed, April 24, 1933.

FIG. 4. Leaves of "white bud" corn plant compared with healthy.

A. Reddish bronze color over entire leaf, green color practically dissipated.

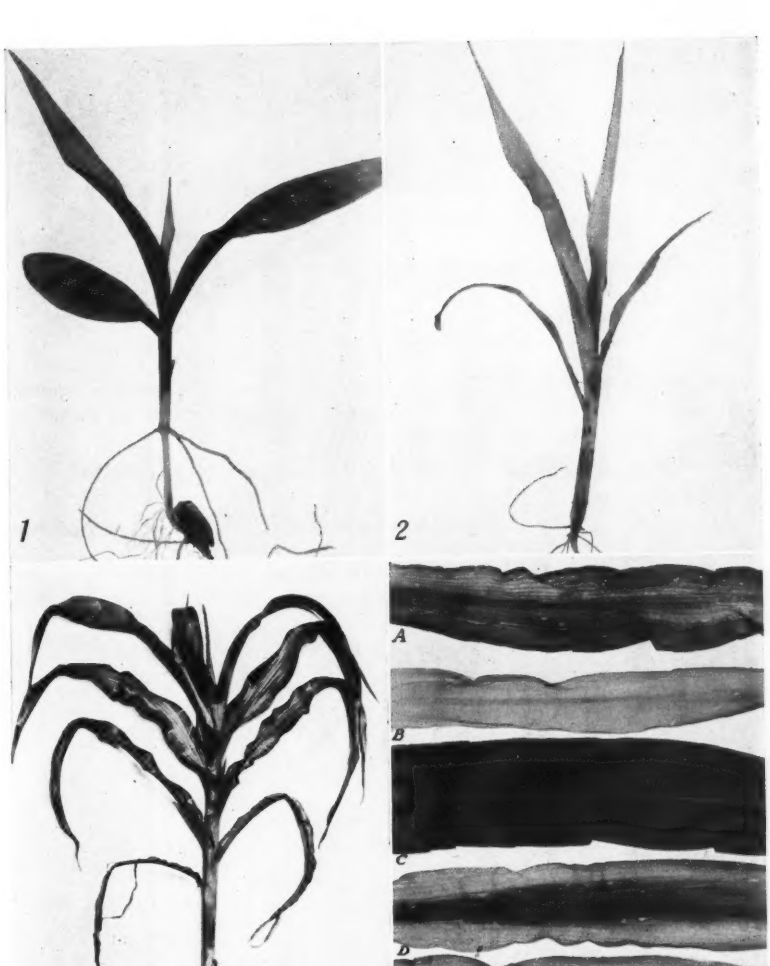
B. Light slate-colored leaf practically dead.

C. Healthy leaf.

D. Green about midrib with light slate-colored edges.

E. Dead straw-colored leaf depleted of chlorophyll with exception of green streaked midrib.

Corn planted, March 3, 1933, photographed, April 24, 1933.



FIGS. 1–4

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PLATE 2

EFFECT OF ZINC SULFATE IN COMBINATION WITH MIXED INORGANIC FERTILIZER AND WITH ALKALINE PEAT ON DEVELOPMENT OF THE CORN PLANT ON SOIL AREAS WHICH PRODUCE "WHITE BUD" OR CHLOROTIC PLANTS

Whatley's Prolific corn, planted April 7, 1933, photographed, June 22, 1933

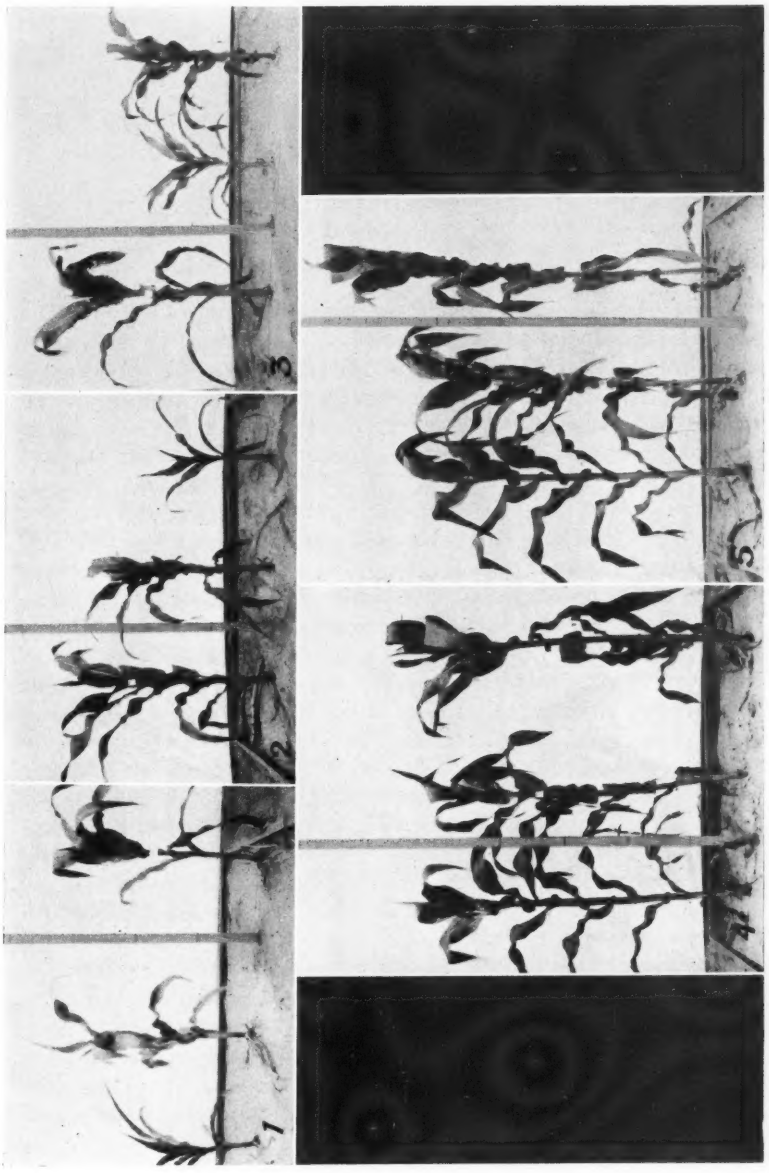
FIG. 1. No treatment.

FIG. 2. Four hundred pounds per acre of a 8–8–4 mixed inorganic fertilizer made from nitrate of soda, superphosphate, and muriate of potash (in row).

FIG. 3. Five tons per acre of alkaline peat in the furrow.

FIG. 4. Four hundred pounds per acre of a 8–8–4 mixed inorganic fertilizer and 20 pounds per acre of zinc sulfate ($ZnSO_4 \cdot 7H_2O$) (in row).

FIG. 5. Five tons per acre of alkaline peat in the furrow with 20 pounds per acre of zinc sulfate ($ZnSO_4 \cdot 7H_2O$).



FIGS. 1–5

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THE LAWS OF SOIL COLLOIDAL BEHAVIOR: XVI. THE CATION EXCHANGE-MAXIMUM IN ALUMINO-SILICATES[1]

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The work on the isoelectrically precipitated alumino-silicates previously reported (1, V) showed that the cation exchange capacity increased with the proportion of silica in the complex. Aluminum hydroxide, isoelectric at pH 8.1, did not show any cation exchange at pH 7.0, and silica gel possesses this capacity to a very moderate extent. It was obvious, therefore, that the power to adsorb and exchange cations must show a maximum at a certain proportion of silica to alumina.

In the isoelectric precipitation, which was done in highly dilute systems (1, III), the alumina would not carry down over 3 mols of SiO_2 per mol Al_2O_3. In more concentrated solutions any amount of silica in excess of this ratio can be forced to precipitate with the alumina, but then the gel always remained electronegative down to the lowest pH employed. At low pH the alumina redissolves. It is apparently not possible to continue the isoelectric series beyond 3 mols SiO_2 per mol Al_2O_3. We have therefore continued the series by precipitation from more concentrated solutions and at a pH high enough to insure complete precipitation of the alumina. The procedure was as follows:

Constant volumes of a 0.1 *M* Na_2SiO_3 solution were rapidly mixed with various quantities of a 0.1 *M* $AlCl_3$ solution containing sufficient HCl to give a final pH between 5.0 and 5.5 as checked by methyl red. The mixture was filtered, and the precipitates were allowed to become air dry. They were then leached with neutral, *N* Ba-acetate until the filtrate was neutral, then treated with 10 cc. *N* $BaCl_2$ and washed until the Cl reaction disappeared. The Ba was then displaced by *N* NH_4Cl and the Ba determined in the usual way. The precipitates were then analyzed for SiO_2 and Al_2O_3. The adsorbed Ba could thus be calculated per millimol SiO_2. The results are given in table 137, which shows (*a*) the number of millimols of SiO_2 and Al_2O_3 in each precipitate; (*b*) the molar percentage (mols SiO_2 + mols Al_2O_3 = 100), (*c*) the molar ratio SiO_2/Al_2O_3, and (*d*) the number of milliequivalents adsorbed Ba in the total precipitate as well as per millimol SiO_2.

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[3] Now at University of Budapest, Hungary.

The exchange capacity previously (1, V) found for silica gel prepared from sodium silicate is included for comparison. Figure 39 shows the relationship more clearly.

The results show a clear maximum in the cation exchange capacity at the ratio of 9 mols of SiO_2 to 1 mol Al_2O_3. In a letter from A. J. Pugh who, together with M. S. duToit, has continued this work at the New Jersey Agricultural Experiment Station, we are informed that he has found the corresponding maximum in a ferric silicate series at a ratio $SiO_2/Fe_2O_3 = 4.2$. The comparison is of interest, for it might help us to explain the cause of the phenomenom.

Mr. Pugh merely states that the maximum for the ferric silicate series was found at the molar ratio of 4.2. The values for the exchange capacities of the

TABLE 137

The cation exchange capacity in alumino-silicates of various molar ratios

MILLIMOLS IN PRECIPITATE		IN 100 MOLS		$\frac{SiO_2}{Al_2O_3}$	Ba ADSORBED	
SiO_2	Al_2O_3	Mols SiO_2	Mols Al_2O_3		Total	Per m. mol SiO_2
					m.e.	*m.e.*
16.65	4.35	79.4	20.6	3.8	1.70	0.102
16.27	3.59	82.0	18.0	4.5	1.76	0.108
16.94	3.01	85.0	15.0	5.6	1.98	0.117
16.66	2.40	87.5	12.5	6.9	2.30	0.138
17.57	2.31	88.3	11.7	7.6	2.52	0.143
15.49	1.78	89.8	10.2	8.7	2.19	0.142
17.01	1.86	90.2	9.8	9.1	2.61	0.153
16.04	1.47	91.7	8.3	10.9	1.93	0.120
16.82	1.50	91.9	8.1	11.2	2.20	0.131
16.17	1.27	92.8	7.2	12.7	1.69	0.104
16.23	0.80	95.4	4.6	20.3	1.30	0.080
15.59	0.68	96.0	4.0	22.9	1.23	0.079
Silica gel.....						0.016

series not being known to us, we are unable to plot the corresponding curve. But the actual position of the two legs of the curve as obtained by Pugh and duToit might be of doubtful value as far as a comparison with our curve representing the aluminum silicate series is concerned, because the properties of colloidal precipitates are often very much affected by the method of precipitation as well as by later treatment. A comparative study can therefore be made only by a strict adherence to a uniform procedure. If we assume, however, that the maximum exchange capacity in the ferric series is equal to that of the aluminum series then we have, together with the two points of origin, the three cardinal points on the ferric silicate curve. On this basis we have plotted a curve (broken line) in our figure merely to indicate the position of the maximum in exchange capacity in the ferric silicate series. (A ratio of 4.2 mols SiO_2 to

1.0 mol Fe_2O_3 corresponds on the basis of molar percentages to 80.8 mols SiO_2 and 19.2 mols Fe_2O_3.)

In the isoelectrically precipitated series it was found: (*a*) that the ferric silicates (as well as the phosphates and humates) possess a lower isoelectric point and a higher cation exchange capacity than the aluminum compounds of the same molar ratios; (*b*) that at the same isoelectric point the ferric complex contains less silica than the aluminum complex; (*c*) that ferric hydroxide has a lower isoelectric point than aluminum hydroxide; and (*d*) that ferric hydroxide when precipitated with bentonite reduced the cation exchange capacity of the latter to a much lesser degree than aluminum when so precipitated.

This could all be satisfactorily explained by the fact that $Fe(OH)_3$ is a weaker base than $Al(OH)_3$. The weaker base leaves a stronger acid residue, and this means a lower isoelectric point and a higher cation exchange capacity.

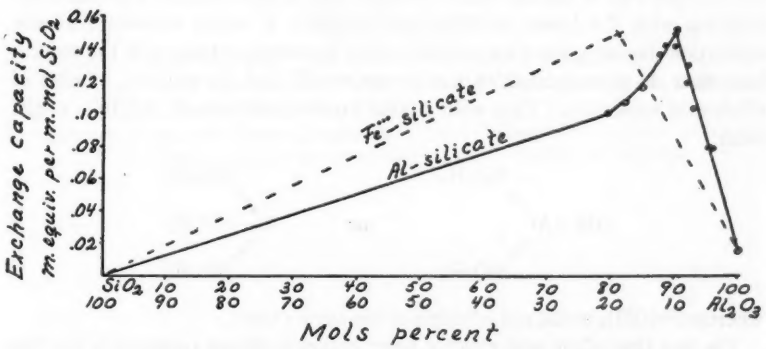


FIG. 39. THE CATION EXCHANGE—MAXIMUM IN PRECIPITATED ALUMINO- AND FERRIC-SILICATES

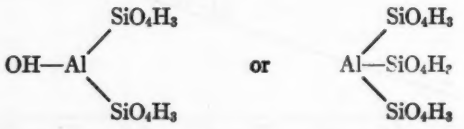
How are we now going to relate this difference in behavior to the two maxima in the cation exchange capacities?

The facts are: (*a*) that silica gel possesses a very moderate cation exchange capacity; (*b*) that in the presence of aluminum and ferric hydroxides (and also, as will be shown in a later publication, in the presence of magnesium) this capacity is substantially increased; and (*c*) that less of the stronger base $Al(OH)_3$ than of the weaker base $Fe(OH)_3$ is required to attain a maximum exchange (compare the right legs of the curves), but that beyond these points, that is, in higher proportions of the bases, the stronger base $Al(OH)_3$ suppresses the exchange power of the silicic acid more than the weaker $Fe(OH)_3$ (compare left legs of the curves).

It appears, therefore, that silicic acid is activated when in partial combination with a base, but that its activity or exchange capacity is again suppressed when the combination with the nondisplaceable cations of the base is more

complete. The fact that the isoelectrically precipitated ferric silicates (also phosphates and humates) possess a higher cation exchange capacity and a lower isoelectric point than the aluminum compounds of the corresponding molar ratios was explained on the theory that the acid residue is stronger in the ferric complex because $Fe(OH)_3$, being a weaker base, ties up a smaller number of acid valencies than $Al(OH)_3$. This we might indicate as follows:

This relationship applies to the left legs of the curves in figure 39 where the proportion of sesquioxide is high. But it can equally well be applied to account for the right legs of the curves, for, if silicic acid is activated by a partial combination with the bases $Al(OH)_3$ and $Fe(OH)_3$, it seems obvious that the activation should proceed more rapidly with the stronger than with the weaker base since, in given quantities, the former would bind the greatest number of silicic acid molecules. Thus with a great excess of silicic acid, $Al(OH)_3$ might form:



whereas $Fe(OH)_3$ would not combine to the same extent.

The fact that silicic acid yields a lower cation exchange capacity in the free condition than in the presence of certain bases might be accounted for if we assume that silicic acid is a pseudo acid which exists chiefly in its non-acidic form. In partial combination with a base with which a practically non-dissociated compound (an acid salt) is formed, a reversion into this non-acidic form would not take place. This would increase the *capacity* to combine with the displaceable cations. Its *strength* as an acid in the free condition appears, however, to be as great as, or greater than, in the partially combined condition because electrodialyzed silica gel showed a lower pH than any of a series of electrodialyzed alumino silicates (2, X).

Since the maximum in the cation exchange capacity must be assumed to coincide with a maximum in the acidic residue, it follows, by application of the aforementioned reasoning, that the maximum for the weaker base must lie at a lower molar ratio SiO_2/R_2O_3 than that of the stronger base. It requires, in other words, more of the weaker base to give rise to a maximum in acidic residue. With more base than corresponds to this maximum the acidic residue becomes neutralized and reduced (more by the stronger than by the weaker

base). With more silica than that which corresponds to this maximum the acidic residue is reduced through a dilution by the less active free silica.

We can find no stoichiometric explanation for the fact that the maximum in the cation exchange capacity was found at a molar ratio of 4.2 in the ferric silicate series and at about 9.0 in the aluminum silicate series. But should a stoichiometric relationship be expected in compounds so complex that each reaction unit contains thousands or millions of molecules and in which the surface composition may differ greatly from the composition of the total gel?

SUMMARY

The maximum in the cation exchange capacity of alumino-silicates has been found to be at a molar ratio of about 9 mols SiO_2 to 1 mol Al_2O_3. A comparison is made with the corresponding ratio (4.2:1) of ferric silicates, and an explanation is offered on the basis of the general chemical and electrokinetic behavior of the two series.

REFERENCES

(1) MATTSON, SANTE 1930–1931 The laws of soil colloidal behavior: III. Isoelectric precipitates. *Soil Sci.* 30: 459–495 (1930); V. Ion adsorption and exchange. *Soil Sci.* 31: 311–331 (1931).

(2) MATTSON, SANTE, AND HESTER, JACKSON B. 1932 The laws of soil colloidal behavior: X. Exchange neutrality and combining capacity. *Soil Sci.* 34: 459–483.